MOLECULAR DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF1-SUBSTITUTED TETRAHYDROPYRIMIDINE DERIVATIVES BY LEUCKART REACTION

Dissertation submitted to

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In partial fulfilment of the requirement
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APRIL-2014

Prof. Dr. A. ABDUL HASAN SATHALI, M.Pharm., Ph. D.,
Principal I/C,
College of Pharmacy,
Madurai Medical College,
Madurai – 20.

CERTIFICATE

This is to certify that the dissertation entitled**" MOLECULAR DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF 1-SUBSTITUED TETRAHYDROPYRIMIDINE DERIVATIVES BY LEUCKART REACTION"** was done by **Ms. R.ELAVARASI, (Reg.no:261215752)** in the Department of Pharmaceutical Chemistry, College of Pharmacy, Madurai Medical College, Madurai-20, in partial fulfillment of the requirement for the Degree of Master of Pharmacy in pharmaceutical Chemistry under guidance and supervision of **Prof. (Mrs.).R.THARABAI, M.Pharm,** HOD, Department of Pharmaceutical chemistry during the academic year 2013-2014.

This dissertation is forwarded to the Controller of Examination, The Tamil Nadu Dr.M.G.R. Medical University, Chennai.

Station: **Dr. A. Abdul Hasan Sathali**, **M.Pharm., Ph.D.,**

Date:

Prof. (Mrs.) R.THARABAI, M.Pharm.,
Head of the Department of Pharmaceutical Chemistry,
College of Pharmacy,
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Station: Madurai **Prof. (Mrs.).R.Tharabai, M. Pharm,**

Date:

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LIST OF ABBREVIATIONS

^{0}C	: Degree centigrade
µg	: Microgram
%	: Percentage
ml	: Milliliter
1H-NMR	: Proton nuclear magnetic resonance
IR	: Infrared
DMSO	: Dimethylsulfoxaide
TLC	: Thin Layer Chromatography
Ar	: Aromatic
Ppm	: Parts per million
Rf	: Retention factor
C	: Carbon
H	: hydrogen
N	: nitrogen
O	: oxygen
Cl	: chlorine
H.A	: Hydrogen acceptors
H.D	: Hydrogen bond donor
mmol	:Milli mole

INTRODUCTION

1. INTRODUCTION

Medicinal chemistry is interdisciplinary subject involving organic chemistry, Inorganic chemistry, Biochemistry, Physiology, Microbiology, Biology, Toxicology and Computer modeling in the research for better and new drug discovery.

Generally medicinal chemistry is used to make new compound, determine its biological efficacy and alter the structure of the compound for optimum effect.

Early drug design started with elucidation of structure in natural products followed by selective changes in the molecule. The later was done for many reasons, including the reduction of any undesirable side effect & to obtain better pharmacokinetic response.

Modern drug design deals with the synthesis of new structure or by making changes in the existing compound and see what happen. This is a fairly recent discipline which is still in its infancy.

The techniques of molecular graphics and computational chemistry have provided novel chemical structure that have led to new drugs with potent medicinal activates.

Medicinal chemistry covers the following stages:

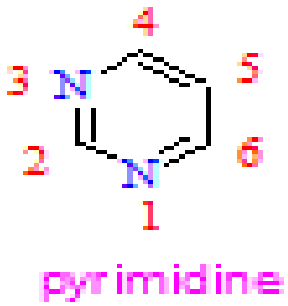
i) In the first stage new active substance or drugs are identified and prepared from natural sources, organic chemical reaction. They are known as lead molecules.

ii) The second stage is optimization of lead molecule to improved potency, selectivity and to reduced toxicity.

Iii) The next stage involves the optimization of synthetic route for bulk production and modification of pharmacokinetic and pharmaceutical properties of active substance to perform it clinically useful.

A. PYRIMIDINE[3,50]

- Pyrimidine is an important heterocyclic molecule is associated with several biological activities. Pyrimidines are heterocyclic compound that have an atom of nitrogen at 1'st and 3'nd position.



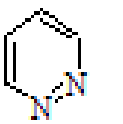
pyrimidine

- Pyrimidine is the most important among the three isomeric diazines.

i)o-diazine

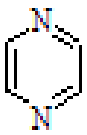
ii)m-diazine

iii)p-diazine



pyridazine

pyrimidine



pyrazine

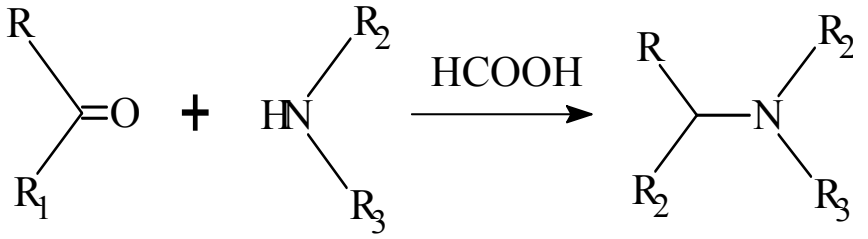
- Pyrimidine is the useful intermediate for the development of many chemotherapeutic agents. It is one of the main pyrimidine nucleuses in anticancer and antiviral agent.
- Various potent drugs in market contains pyrimidine nucleus like pyrantel pamoate (anthelmintic), flucytosine (antifungal), minoxdil (antihypertension), fluorouracil and

floxuridine (antineoplastic), pyrimethamine (antimalarial), idoxuridine and trifluridine (antiviral).

- The important pyrimidine compounds have diverse applications as bactericidal, fungicidal, analgesic, anti inflammatory, anticancer, antiviral, antimalarial, anthelmintic, antihypertension etc.
- Pyrimidine ring is also found in vitamin and barbituric acid.

LEUCKART REACTION[3]

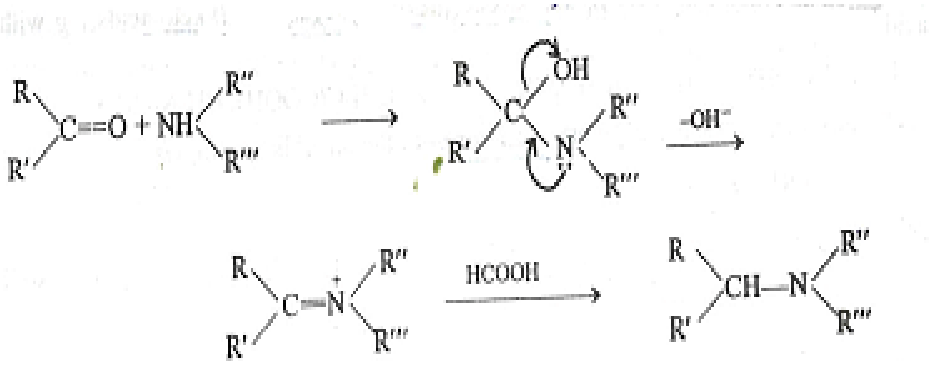
- This reaction is reductive amination process which converts primary or secondary amine to tertiary amine using protonated ketone and formic acid. This reaction avoids the problem of quterization.



Mechanism:

- Amine reacts with protonated ketone to give iminium ion.
- The iminium ion then react with formic acid to give methylated ammonium ion and release CO_2 gas, where formic acid act as a reducing agent or hydride transfer reagent.
- This CO_2 gas leads the synthesis process to the next level of synthesis.
- In this stage ammonium ion gets deprotonated to form final methylated amine product.

- If reaction occurs with primary amine same process follows twice to reach the tertiary amine as a final product.



B. ANTIOXIDANT[54]

Anti-oxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent.

Oxidation reaction can produce free radicals. In turn, these radicals can start chain reaction. When the reaction occurs in a cell, it can cause damage or death to the cell. Anti-oxidants terminate this chain reaction by remove free radical intermediates, and inhibit other oxidation reaction. So anti-oxidants are often reducing agents such as thiols, ascorbic acid or poly phenols. Ascorbic acid and tocopherols are antioxidant.

Antoxidant has been reduced risk factor such as aging, cancer, inflammatory disease, diabetes, heart diseases.

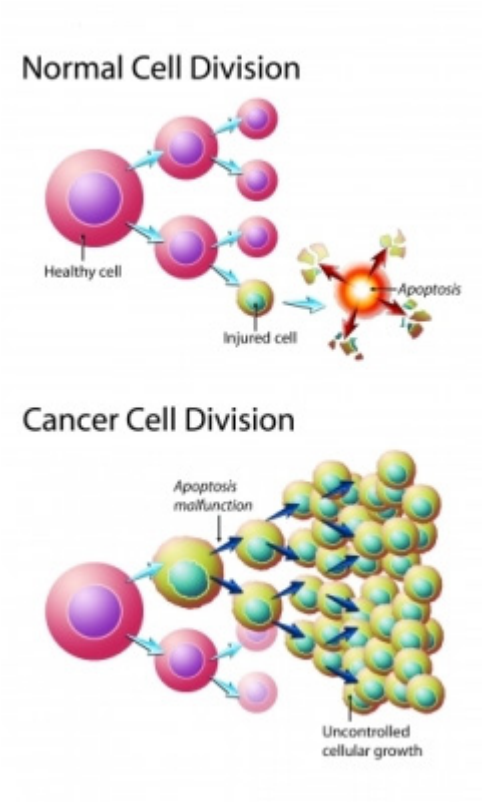
C. ANTICANCER DRUGS[1]

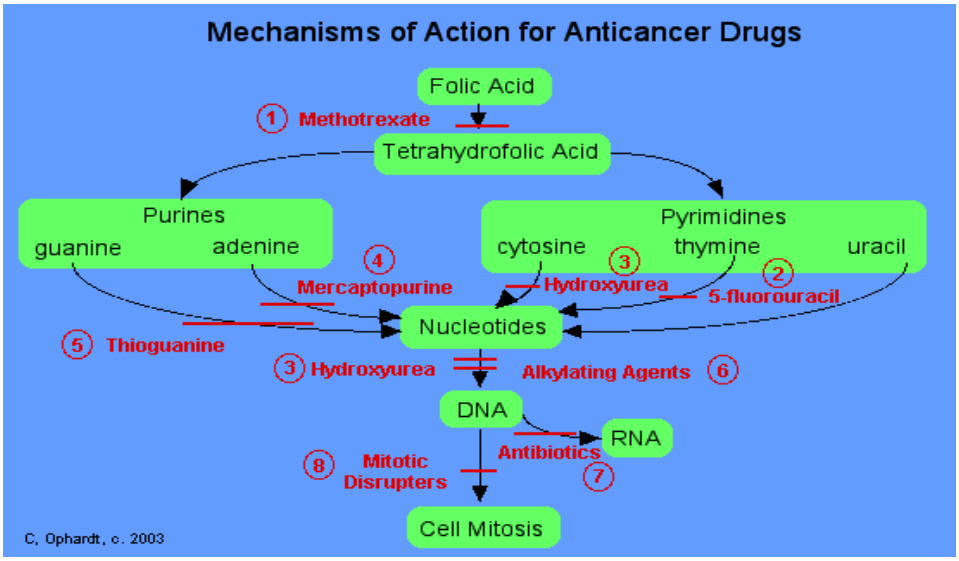
Cancer is defined as a group of diseases which are characterized by uncontrolled cell proliferation and subsequent growth of abnormal tissue leading to profound changes in physiological function. Cancers can arise from both genetic and lifestyle factors that lead to abnormal regulation in the growth of particular stem cell populations. Anticancer or antineoplastic agents are the drugs used in the treatment of cancer, malignancy, tumour, carcinoma, sarcoma, leukemia etc.

Two key aspects of cellular life are i) DNA synthesis and mitosis to produce new cell and ii) cell differentiation which produces specialized cells.

Normal cell have control mechanisms to modulate these two processes by growth factor or growth inhibitor. A balance between cell growth and cell death is maintained, cell death is actively regulated by process known as "apoptosis". Apoptosis is defined as a process of cell shrinkage, membrane blabbing and nuclear condensation.

Cancer cell, this regulatory process is aberrant; they produce over production of growth factor and avoid apoptosis which continue to multiply in an unregulated manner. The unregulated growth causes damage to DNA, resulting in mutations to genes that encode from protein controlling cell division.


Normal Cell Division
Healthy cell
Injured cell
Apoptosis
Cancer Cell Division
Apoptosis malfunction
Uncontrolled cellular growth


Mechanisms of Action for Anticancer Drugs
Folic Acid
1 Methotrexate
Tetrahydrofolic Acid
Purines
guanine
adenine
Pyrimidines
cytosine
thymine
uracil
4 Mercaptopurine
3 Hydroxyurea
2 5-fluorouracil
Nucleotides
5 Thioguanine
3 Hydroxyurea
Alkylating Agents 6
DNA
RNA
Antibiotics 7
8 Mitotic Disrupters
Cell Mitosis
C. Ophardt, c. 2003

D. ANTMICROBIAL DRUGS[6]

The control of microorganism is essential for the prevention and treatment of diseases, microorganism also grow on and with in other organism, and how ever the microbial colonization can lead to disease, disability and even death. Thus the Control or destruction of micro organisms inside the human beings or other animals is great of importance.

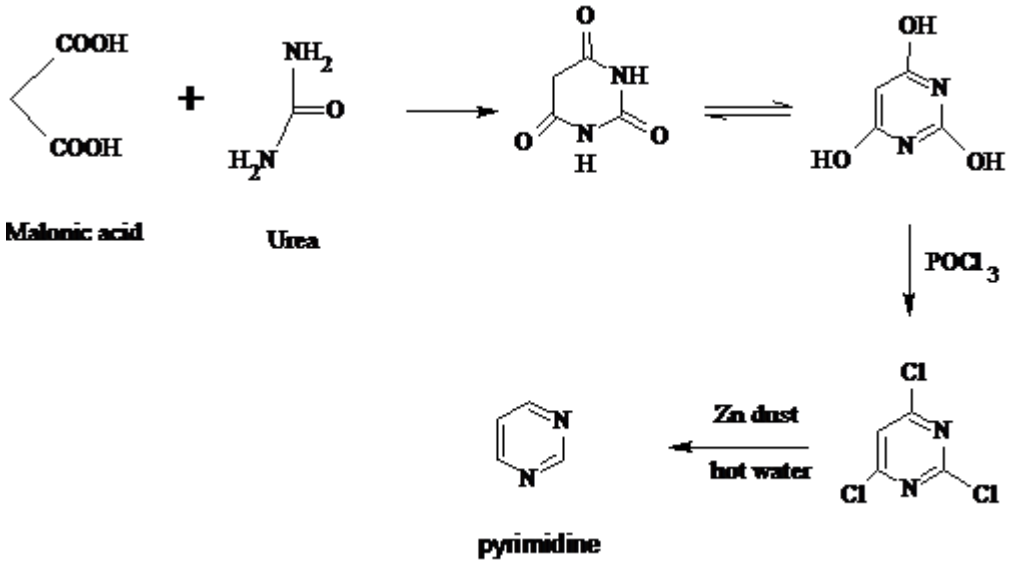
The Different chemical substances excreted by some micro organism, which inhibit the growth and development of other microbes it is called as antibiotics. Some of these drugs that are obtained naturally were put to chemical modifications in an attempt to enhance the beneficial effects mean while minimizing the toxic effects.

The resultant modified product is termed as semi synthetic antibiotics, most of the antibiotics currently used are semi synthetic The chemists have synthesized many drugs that have got the antibacterial property and less toxicity, these drugs are called synthetic antibiotic drugs and it is further divided into,

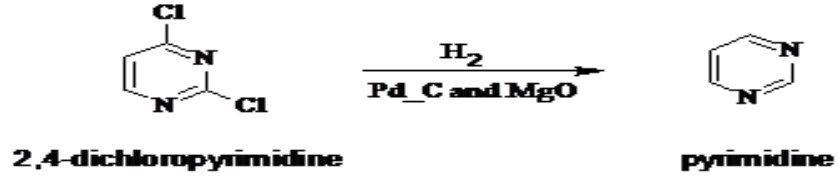
1. Anti bacterial drugs.
2. Anti viral drugs.
3. Antifungal drugs.
4. Anthelmintic drugs.

E. SYNTHESIS OF PYRIMIDINE AND ITS DERIVATIVE[3]

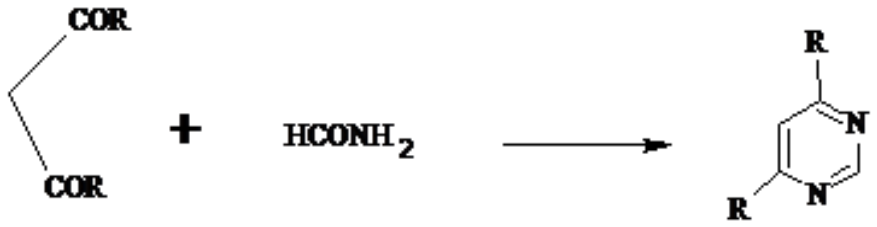
- **Gabrial synthesis:** Pyrimidine is prepared from barbituric acid which in turn can be obtained from malonic acid and urea.



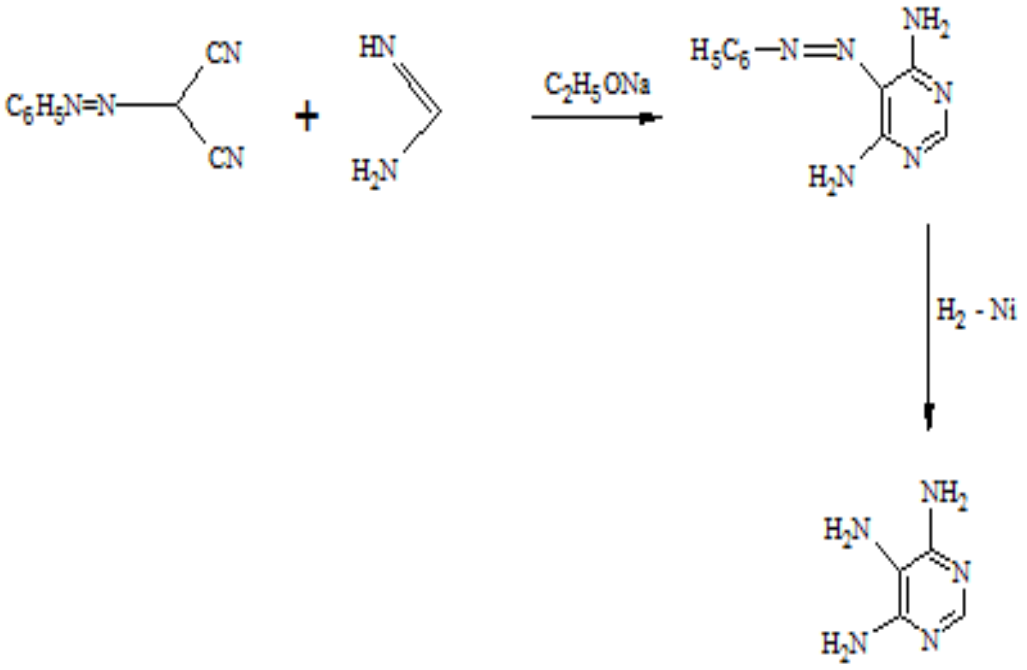
- **Whittaker syntheis:** Pyrimidine is prepared by catalytic reductive dechlorination of 2, 4 –dichloropyrimidine.



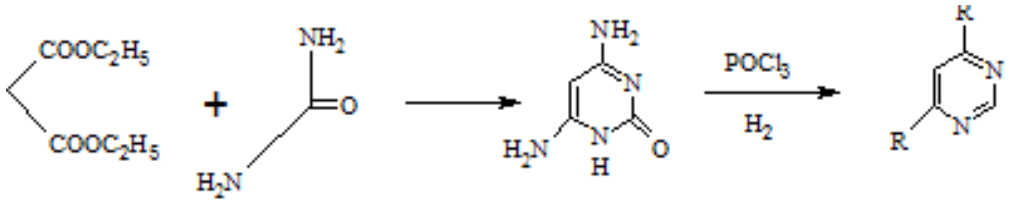
- Pyrimidine is prepared by condensation of β-diketones with formamide at 180-200°C.



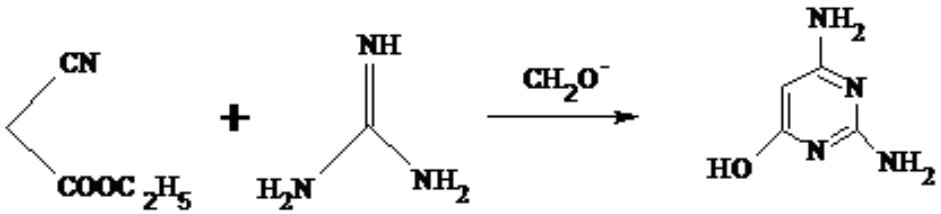
- **Todd synthesis:** 4, 5, 6 triaminopyrimidine is prepared by the condensation of formamide with phenylazomalononitrile.



- Pyrimidine derivative is prepared by condenasation of urea and malonic ester in the presence of POCl3.



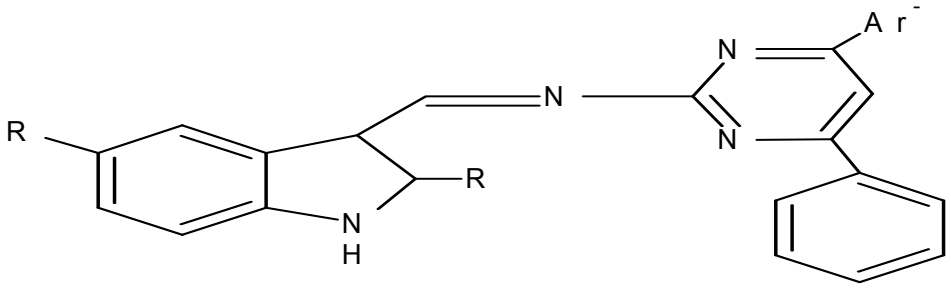
- 2, 4-diamino 6-hydroxypyrimidine is obtained from ethyl cyanoacetate and guanidine in the presence of ethoxide ion.



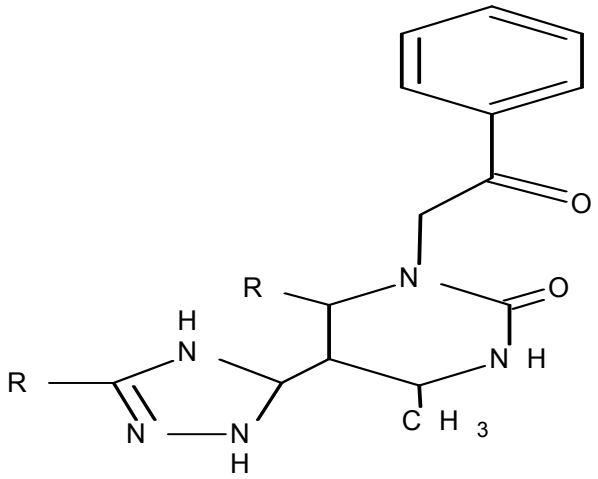
LITERATURE REVIEW

2. LITERATURE REVIEW

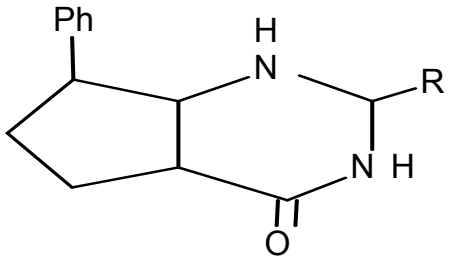
- **PADMASHRI *et al*.,** reported the synthesis of 2-(2', 5'-substituted indole-3'-yl methylene imino)-4, 6–diaryl pyrimidine with a review to screen them for their antimicrobial activity (2002).



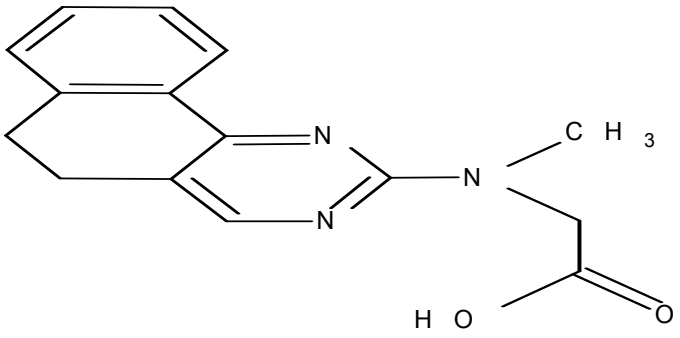
- **MISHRA *et al*.,** synthesized various derivatives of pyrimidine and evaluated their fungicidal activity (2004).



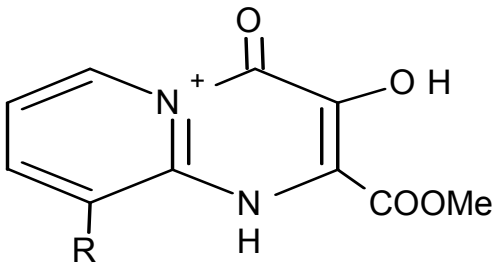
- **CJ SHISHOO *et al*.,** have prepared some substituted 6- phenyl and 7-phenyl thieno (3, 2-d) pyrimidine 4-ones with anti-hyperlipidemic activity (1994).



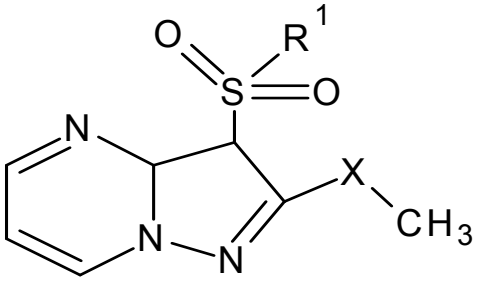
- **B.MURUGESH *et al*.,** have prepared some pyrimidine derivatives and evaluated for analgesic and anti- inflammatory activity (2007).



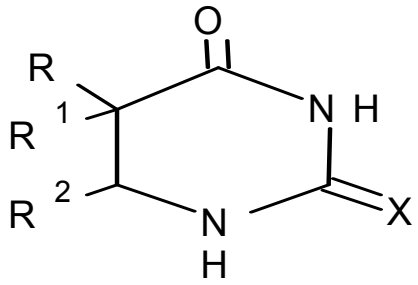
- **M.DAKSHELA *et al*.,** has synthesized and tested for pyrimidine derivatives with anticancer activity (1995).



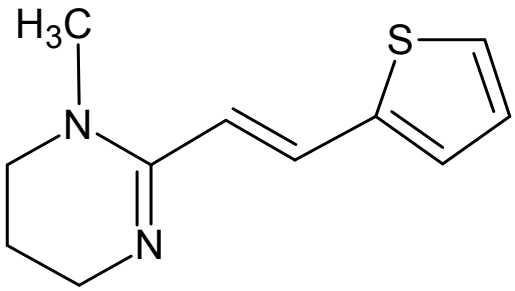
- An efficient and reliable synthesis of pyrimidine derivatives with HIV-I integrase inhibitor (1994).



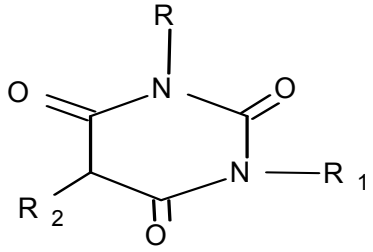
AMIR *et al.*,have prepared some pyrimidine derivatives with hyperthyroidism activity. Thiouracil and its alkyl analogue, thio -barbital are efficient drug against hyperthyroidism (2001).



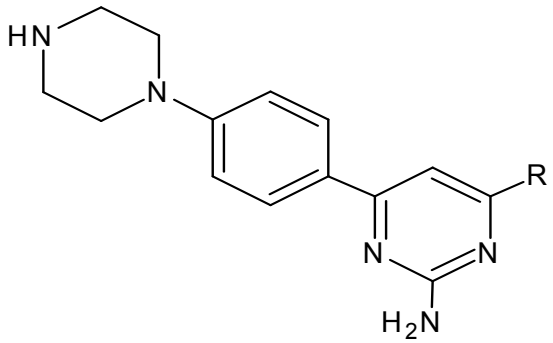
- **LEE *et al.*,** has synthesized some novel pyrimidine derivative having thiozolidinone. These compounds were evaluated for their glucose and lipid lowering activity (2005).
- An efficient and reliable synthesis of pyrimidine derivatives with anthelmintic activity (1990).



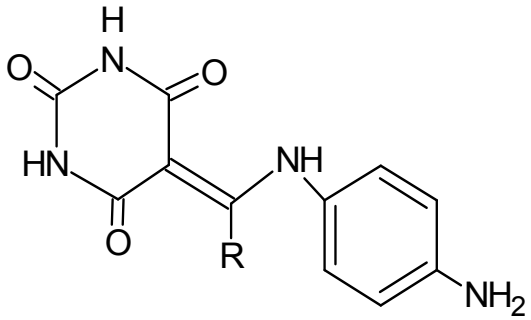
- **T.EUNICE *et al.*,** has synthesized pyrimidine derivatives and evaluated for agents involved in the category includes sedatives, hypnotics, anticonvulsant, anxiolytic activity (1998).



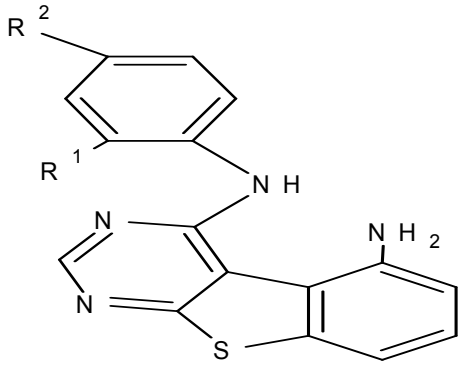
- **E.NIRANJI *et al*.,** Novel pyrimidines were synthesized by the condensation of chalcones of 4'-piperazine acetophenone with guanidine hydro chloride and evaluated for antihistamine activity (2000).



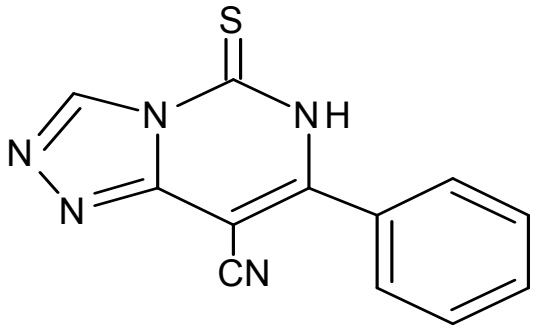
- **PALWINDER SINGH *et al*.,** have reacted 5-benzoyl/5-carbaldehyde/5-(3-phenyl acryloyl)-6-hydroxy -1H-pyrimidine-2 diones with amine provided the corresponding enamines with anticancer activity (1995).



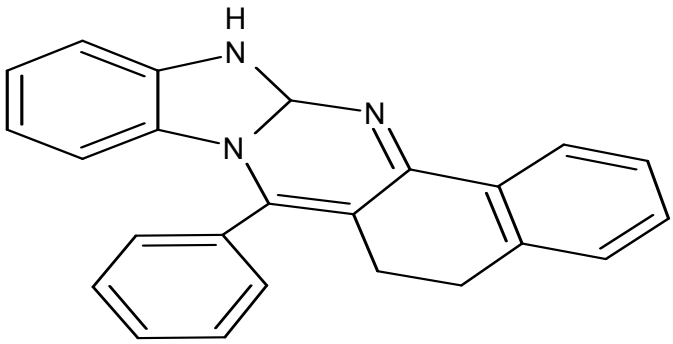
- **STEPHANEPEDE BOSCQ *et al*.,** has synthesized 4-(2-methylanilino) benzothieno (2, 3-d) pyrimidine and 4-(2- methoxyanilino) benzothieno (2, 3-d) pyrimidine which showed as cytotoxic activity (1992).



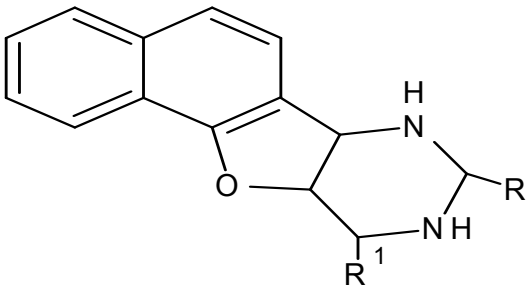
- **FATHALLA et al.,** has synthesized a series of some pyrimidine derivatives with antibacterial and anticancer activity (2009).



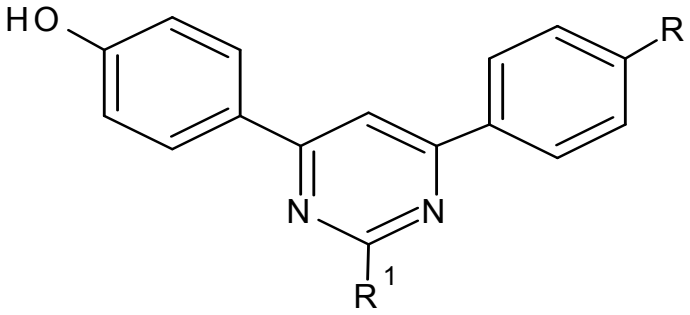
- **DESENKO *et al*.,** has synthesized azolopyrimidine derivatives and compounds were evaluated for hypoglycemic activity (1995).



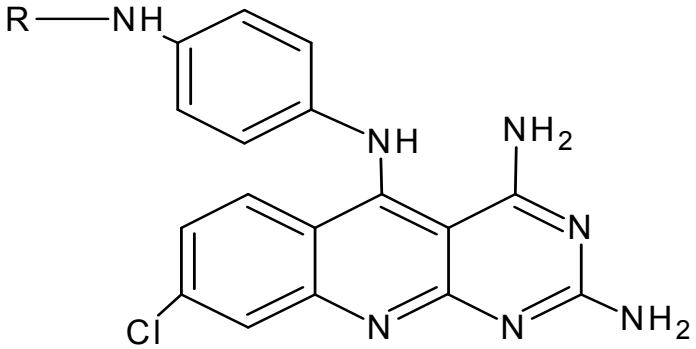
- **PADMA SHALE *et al*.,** have been reported naphtha (2, 1-b) furo (3, 2-d) pyrimidine derivative and evaluated for anti- inflammatory activity (2005).



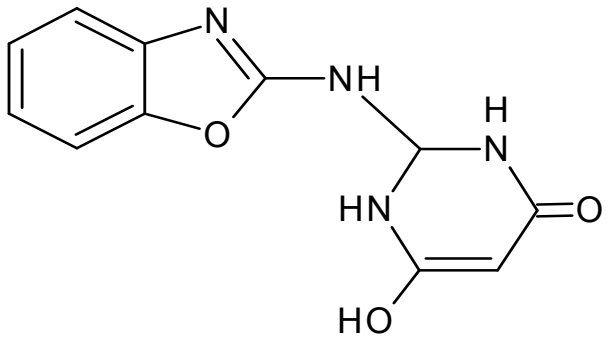
- **ANU *et al*.,** has synthesized tri-substituted pyrimidine derivatives evaluated for their *in-vitro* antimalarial and anti -tubercular activities (1993).



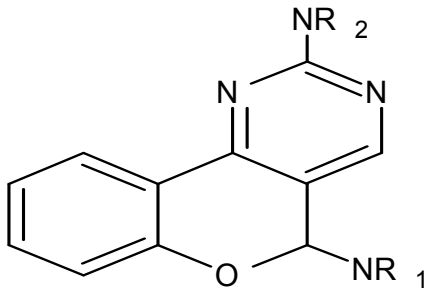
- **VISHWANADHAN *et al*.,** reported the synthesis of some novel 5-substituted amino-2, 4-diamino -8-chloro pyrimido-(4, 5-b) quinolines with antimalarial activity (2005).



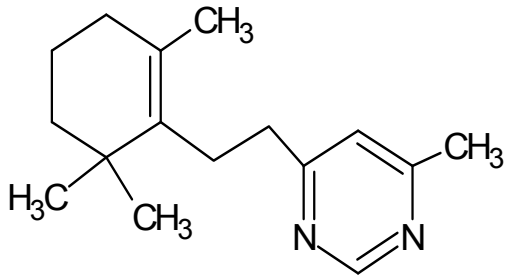
- **SHERIFF *et al*.,** has synthesized 2-(benzoxazole -2-yl-amino)-3H-4-oxopyrimidines and screened for *in-vitro* anti HIV activity (2003)



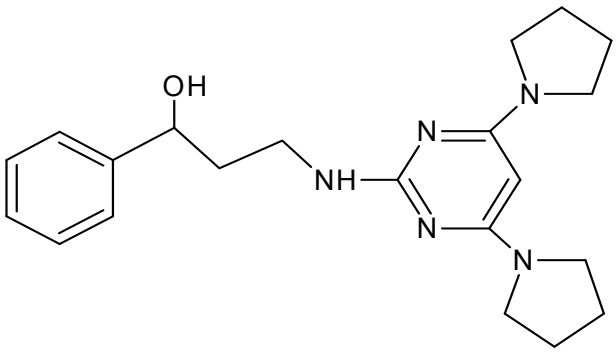
- **BRUNI *et al*.,** reported the synthesis of some new 2, 5-cycloamino 5H-benzopyrano (4, 3-d) pyrimidine which showed anti- inflammatory activity (1993).



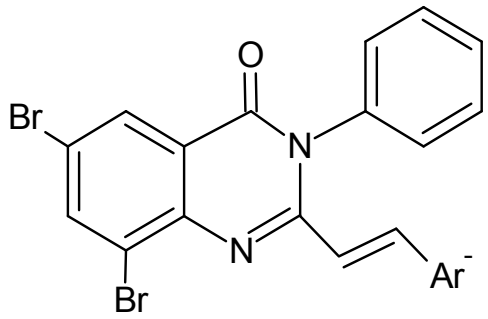
- **PANDEYA *et al*.,** has synthesized some novel terpenylpyrimidines having anti leishmanial activity (2004).



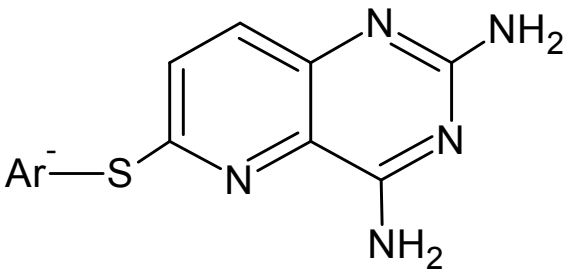
- **JOUBRAN *et al*.,** has synthesized new aryl propanalamines which showed as antioxidant activity and neuroprotective agent (2003).



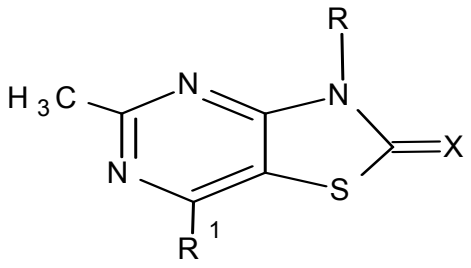
- **MURUGAN *et al*.,** reported the synthesis of certain 2- substituted benzo-pyrimidine and evaluated their anticancer and cytotoxic activity (2004)



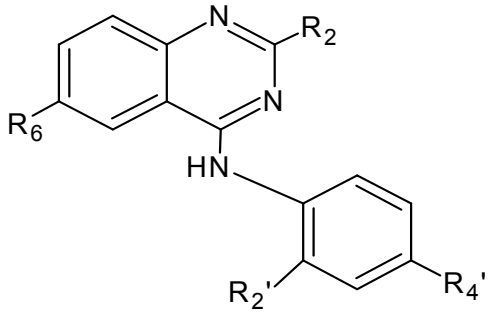
- **WERBEL *et al*.,** has synthesized a variety analogues of 2, 4-diamino 6-(arylthio) quinazolines with antimalarial and antitumour activities (1987).



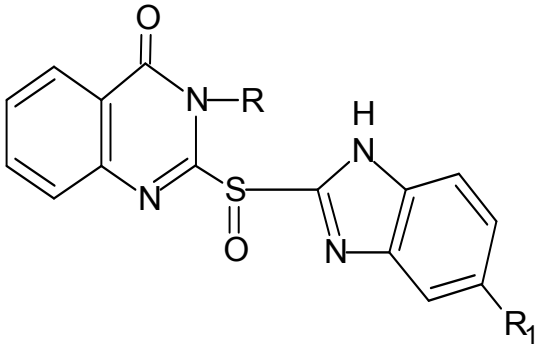
- **KUMARASWAMY *et al*.,** has synthesized and evaluated some angularly fused naphtho (2,1-b) furo (3,2-b) pyrimidines with increase in their diuretic and anti - inflammatory activities (2006)
- **BECK *et al*.,** has synthesized a series of thiazolo (4,5-d) pyrimidine thione and evaluated their antipsychotic activity (2002).



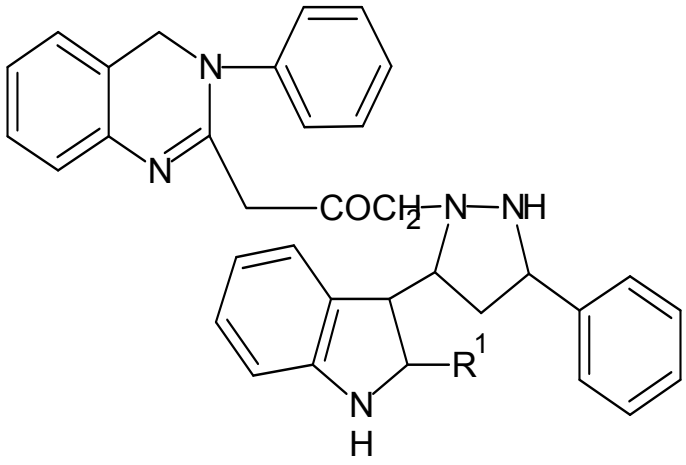
- **GOTTASOVA *et al.*,** reported a series of 2, 6-disubstituted 4-anilino quinazolines and evaluated for antibacterial activity (1990).



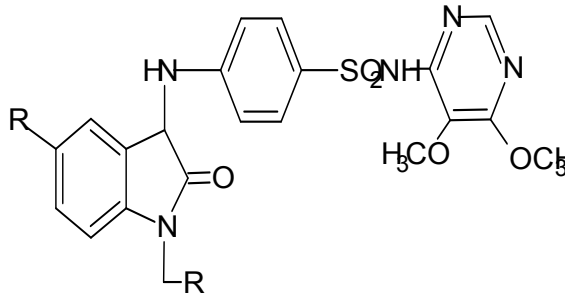
- **AVINASH *et al.*,** has synthesized certain 2-substituted benzopyrimidine and evaluated their cytotoxic activities (2004).



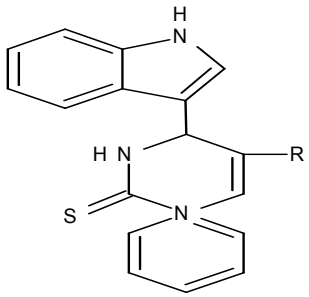
- **ASHOK *et al.*,** has synthesized a series of substituted pyrimidine derivative and tested for anti -inflammatory, analgesic, ulcerogenic activities (1993).



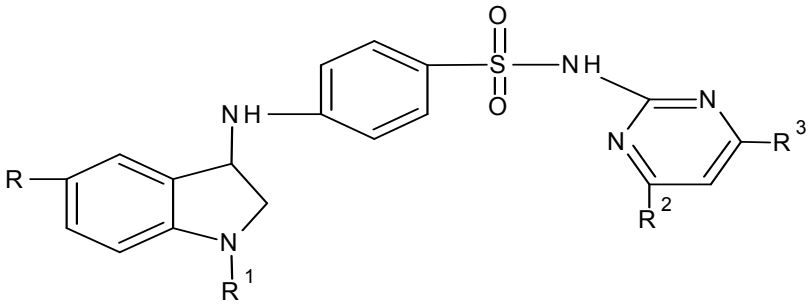
- **PANDEYA *et al.*,** synthesized a series of novel substituted pyrimidine derivatives and evaluated their antimicrobial activity (2004).



- **AMIR *et al.*,** prepared and screened for the biological activities of some 4-(1H-indole-3-yl)-6-phenyl-1, 2, 3, 4-tetrahydro pyrimidine-2-2 one thiones as potent anti-inflammatory agents (2007).



- **RAHAMAN *et al.*,** prepared some derivative of pyrimidine and evaluated their antiviral activity (2009).



SCOPE OF STUDY

AND

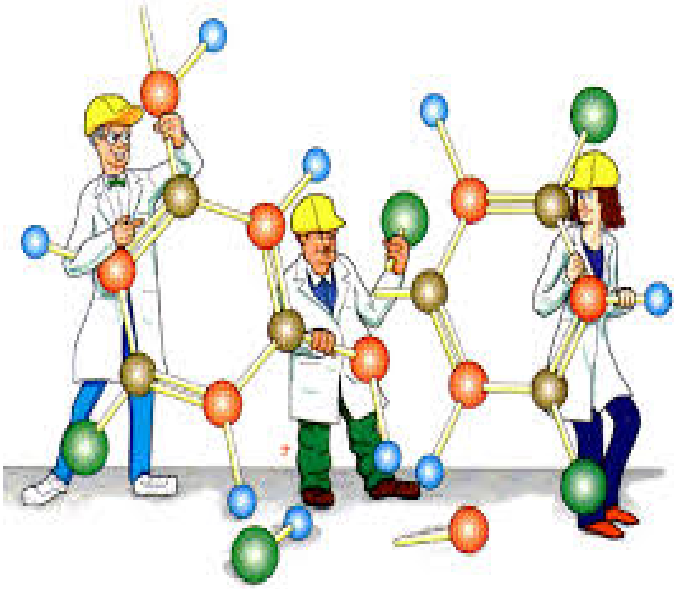
PLAN OF WORK

3. SCOPE OF STUDY

- ✓ Pyrimidine derivatives play a vital role providing exclusive clinical application and minimum toxic level. Recent observation also revealed, pyrimidines are closely related to nucleotides which can easily bind to biomolecules. So interested in selecting this nucleus for my present study.
- ✓ The present study for synthesis of 1-substituted 2-amino-4-oxo -6-aryl 1, 4, 5, 6-tetrahydropyrimidine -5-carbonitrile by Leuckart reaction.
- ✓ These reactions were carried out earlier in the presence of amine, ketones and formic acid. I have introduced different ketones (Acetophenone & Benzophenone) with solvent free reaction condition, having lesser reaction time using Microwave irradiation and yield higher percentage of products.
- ✓ From the literature point of view pyrimidine derivatives display a broad spectrum of biological activities.
- ✓ The present work have been design to carry out synthesis of 1-substituted tetrahydro pyrimidine derivatives followed anticancer , anthelmintic, antimicrobial activities.

PLAN OF WORK

- ✓ To design lead molecule of 2-amino-4-oxo-6-aryl 1,4,5,6-tetrahydropyrimidine -5-carbonitrile by molinspiration, chemdoodle, chemsketch.
- ✓ The compounds were synthesized by Leuckart reaction using Microwave irradiation.
- ✓ These synthesized compounds of 1-substistuted tetrahydropyrimidine derivatives were confirmed using TLC.
- ✓ To carry out the preliminary test such as melting point and solubility.
- ✓ To confirm the structure of synthesized compounds by IR, MASS, ^{1}H NMR spectroscopy.
- ✓ To evaluate the proposed compounds for in-vitro anticancer, antibacterial, antifungal activity



MOLECULAR DESIGN

4. MOLECULAR DESIGN

MOLINSPIRATION

This software is used to calculate the following properties

- ✓ Molecular weight
- ✓ Lipophilicity
- ✓ Bioactivity score

i) GPCR ligand

ii) Ion channel modulator

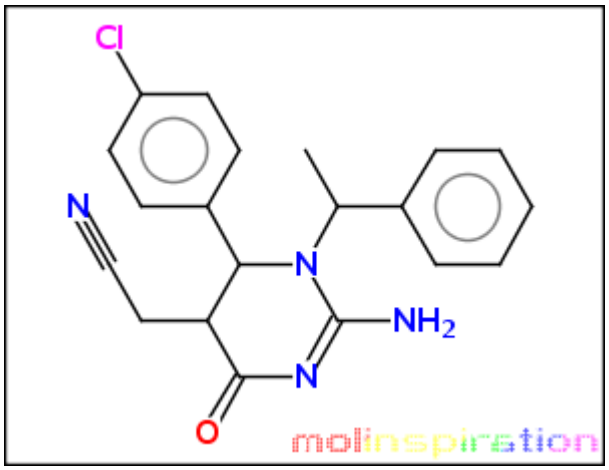
iii) Kinase inhibitor

iv) Nuclear receptor ligand

v) Protease inhibitor

vi) Enzyme inhibitor

D – E1



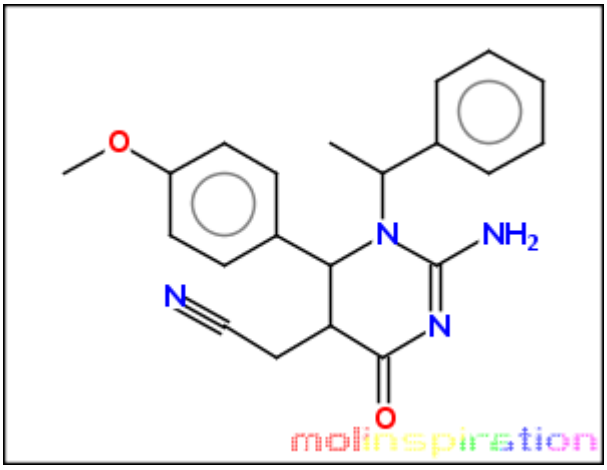
Molinspiration property engine v2013.09

miLogP	2.892
TPSA	82.488
natoms	26.0
MW	366.852
nON	5
nOHNH	2
nviolations	0
nrotb	4
volume	325.168

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.16
Ion channel modulator	-0.32
Kinase inhibitor	-0.50
Nuclear receptor ligand	-0.72
Protease inhibitor	0.09
Enzymeinhibitor	-0.40

D – E2



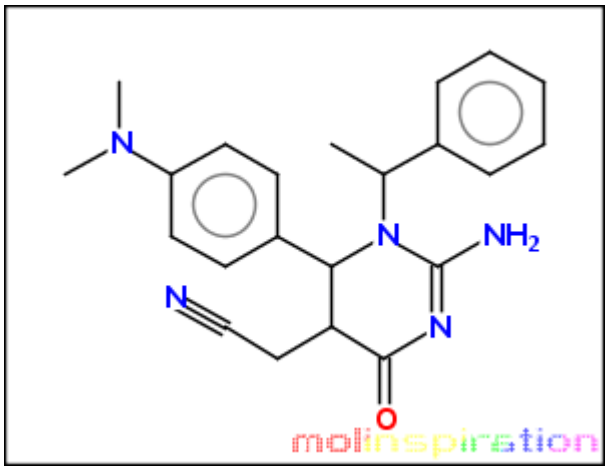
Molinspiration property engine v2013.09

miLogP	2.271
TPSA	91.722
natoms	27.0
MW	362.433
nON	6
nOHNH	2
nviolations	0
nrotb	5
volume	337.178

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.19
Ion channel modulator	-0.37
Kinase inhibitor	-0.50
Nuclear receptor ligand	-0.69
Protease inhibitor	0.07
Enzymeinhibitor	-0.40

D – E3



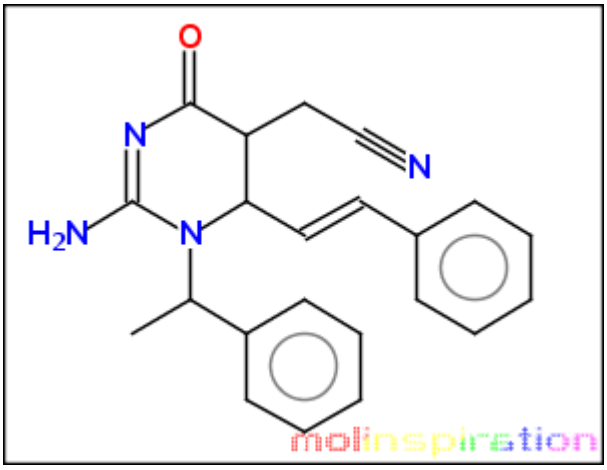
Molinspiration property engine v2013.09

miLogP	2.316
TPSA	85.726
natoms	28.0
MW	375.476
nON	6
nOHNH	2
nviolations	0
nrotb	5
volume	357.538

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.15
Ion channel modulator	-0.31
Kinase inhibitor	-0.41
Nuclear receptor ligand	-0.64
Protease inhibitor	0.09
Enzymeinhibitor	-0.36

D – E4



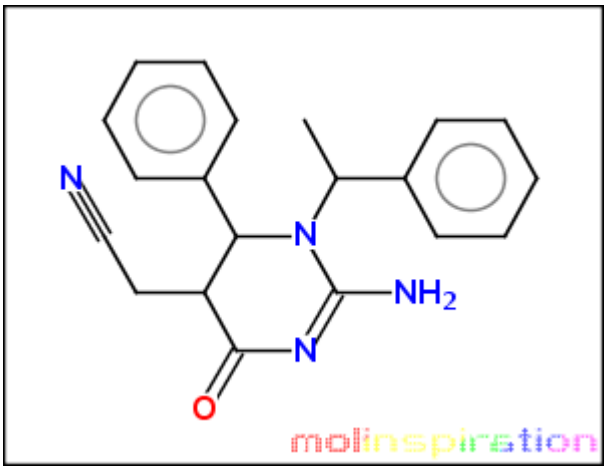
Molinspiration property engine v2013.09

miLogP	2.97
TPSA	82.488
natoms	27.0
MW	358.445
nON	5
nOHNH	2
nviolations	0
nrotb	5
volume	339.049

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.02
Ion channel modulator	-0.19
Kinase inhibitor	-0.29
Nuclear receptor ligand	-0.38
Protease inhibitor	0.39
Enzymeinhibitor	-0.12

D – E5



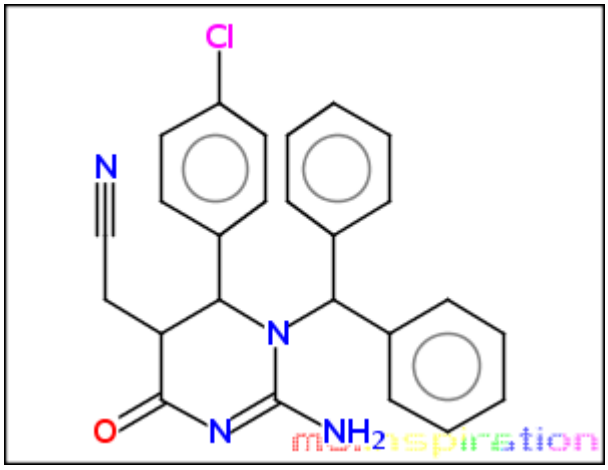
Molinspiration property engine v2013.09

miLogP	2.214
TPSA	82.488
natoms	25.0
MW	332.407
nON	5
nOHNH	2
nviolations	0
nrotb	4
volume	311.632

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.16
Ion channel modulator	-0.32
Kinase inhibitor	-0.50
Nuclear receptor ligand	-0.72
Protease inhibitor	0.14
Enzymeinhibitor	-0.38

D – E6



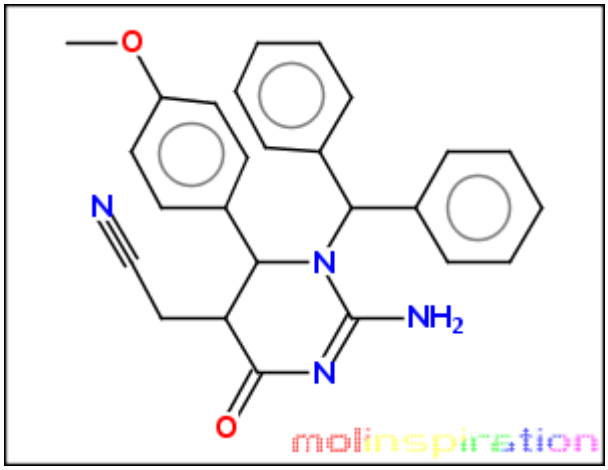
Molinspiration property engine v2013.09

miLogP	4.111
TPSA	82.488
natoms	31.0
MW	428.923
nON	5
nOHNH	2
nviolations	0
nrotb	5
volume	380.016

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.13
Ion channel modulator	-0.23
Kinase inhibitor	-0.49
Nuclear receptor ligand	-0.59
Protease inhibitor	0.01
Enzymeinhibitor	-0.32

D – E7



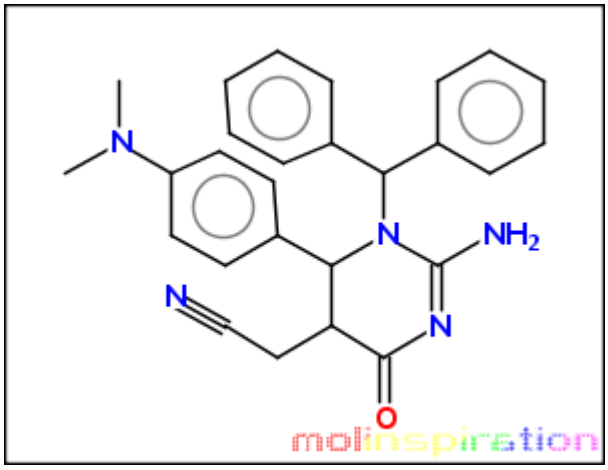
Molinspiration property engine v2013.09

miLogP	3.489
TPSA	91.722
natoms	32.0
MW	424.504
nON	6
nOHNH	2
nviolations	0
nrotb	6
volume	392.025

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.16
Ion channel modulator	-0.28
Kinase inhibitor	-0.49
Nuclear receptor ligand	-0.57
Protease inhibitor	-0.00
Enzymeinhibitor	-0.32

D – E8



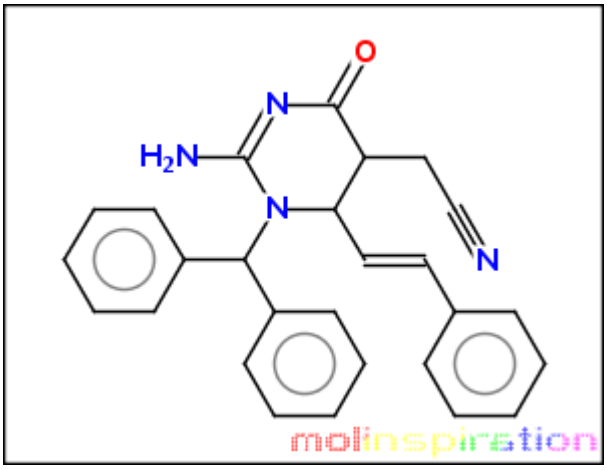
Molinspiration property engine v2013.09

miLogP	3.535
TPSA	85.726
natoms	33.0
MW	437.547
nON	6
nOHNH	2
nviolations	0
nrotb	6
volume	412.386

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.12
Ion channel modulator	-0.24
Kinase inhibitor	-0.42
Nuclear receptor ligand	-0.53
Protease inhibitor	0.01
Enzymeinhibitor	-0.29

D – E9



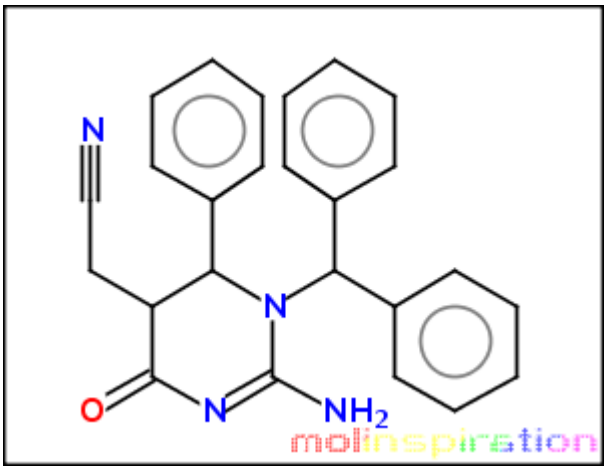
Molinspiration property engine v2013.09

miLogP	4.189
TPSA	82.488
natoms	32.0
MW	420.516
nON	5
nOHNH	2
nviolations	0
nrotb	6
volume	393.896

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.00
Ion channel modulator	-0.11
Kinase inhibitor	-0.29
Nuclear receptor ligand	-0.27
Protease inhibitor	0.29
Enzymeinhibitor	-0.05

D – E10



Molinspiration property engine v2013.09

miLogP	3.433
TPSA	82.488
natoms	30.0
MW	394.478
nON	5
nOHNH	2
nviolations	0
nrotb	5
volume	366.48

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.13
Ion channel modulator	-0.23
Kinase inhibitor	-0.49
Nuclear receptor ligand	-0.59
Protease inhibitor	0.05
Enzymeinhibitor	-0.30

CHEMDOODLE

This software is used to calculate the following properties.

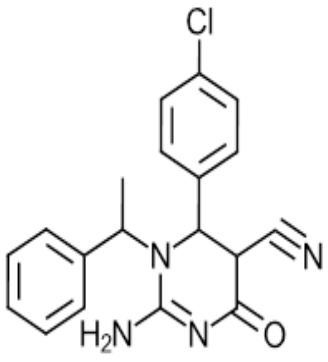
- ✓ Molecular weight
- ✓ Hydrogen bond donor
- ✓ Hydrogen bond acceptor
- ✓ Lipophilicity
- ✓ Molar refractivity

Lipinski's rule:

Lipinski's rule of five states that, in general, an orally active drug has:

1. Not more than 5 hydrogen bond donors.
2. Not more than 10 hydrogen bond acceptors.
3. Molecular weight below 500 g / mol.
4. Partition co-efficient log P less than 5.
5. Molar refractivity values must between 40 -130cm^3/mol

D – E1



2-amino -6-(4-chloro phenyl)-4-oxo -1-(1-phenylethyl)1,4,5,6 -tetrahydro pyrimidine -5- carbonitrile

Molecular Formula = C19H17ClN4O

Molecular Mass = 352.8175

Hydrogen Bond Acceptor Count = 5

Hydrogen Bond Donor Count = 1

Rotatable Bond Count = 3

Lipophilicity (Log P) = 2.8900

Molar Refractivity = 98.9070cm^3/mol

Liquid Viscosity (η_L) = 0.0018N.s/m^2

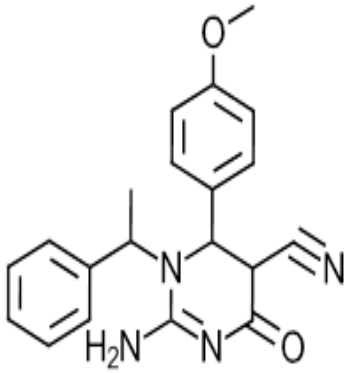
Boiling Point (T_b) = 983.0800K

Freezing Point (T_b) = 543.0800K

Bioavailabilty Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

D – E 2



2-amin-6-(4-methoxy phenyl) -4-oxo -1- (1-phenylethyl) 1,4,5,6 -tetrahydropyrimidine -5-carbonitrile

Molecular Formula =C20H20N4O2

Molecular Mass = 348.9070

Hydrogen Bond Acceptor Count = 6

Hydrogen Bond Donor Count = 1

Rotatable Bond Count = 4

Lipophilicity (Log P) = 3.000

Molar Refractivity = 100.1620cm^3/mol

Liquid Viscosity (η_L) = 0.0016N.s/m^2

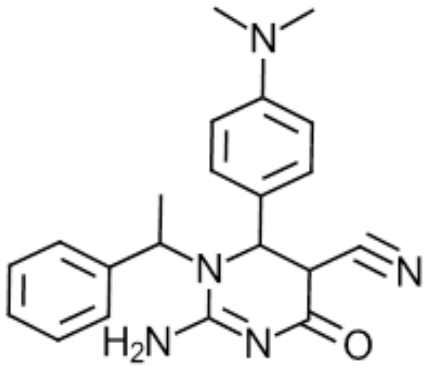
Boiling Point (T_b) = 990.9500K

Freezing Point (T_b) = 546.660K

Bioavailabilty Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

D – E 3



2-amino -6-[4-(dimethyl amino) phenyl] -4-oxo -1-(1-phenylethyl) 1,4,5,6 - tetrahydropyrimidine -5-carbonitrile

Molecular Formula =C20H23N4SO

Molecular Mass = 361.4420

Hydrogen Bond Acceptor Count = 6

Hydrogen Bond Donor Count = 1

Rotatable Bond Count = 4

Lipophilicity (Log P) = 3.300

Molar Refractivity = 106.9560cm^3/mol

Liquid Viscosity (η_L) = 0.0018N.s/m^2

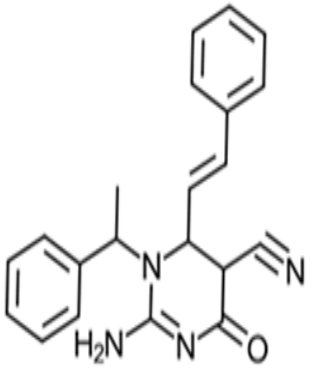
Boiling Point (T_b) = 1003.8500K

Freezing Point (T_b) = 568.1700K

Bioavailabilty Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

D – E4



2-amino -4-oxo [(E)-2-phenylethenyl] -1-(1-phenylethyl)

1,4,5,6-tetrahydropyrimidine -5-carbonitrile

Molecular Formula = C21H20N4O

Molecular Mass = 344.4097

Hydrogen Bond Acceptor Count = 5

Hydrogen Bond Donor Count = 1

Rotatable Bond Count = 4

Lipophilicity (Log P) = 3.22

Molar Refractivity = 103.7560cm^3/mol

Liquid Viscosity ($_{?L}$) = 0.0032 N.s/m^3

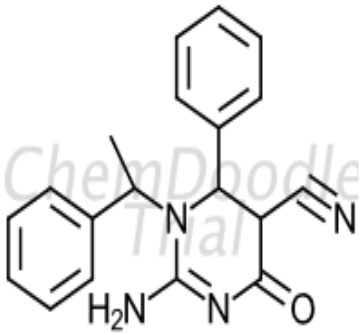
Boiling Point (T_b) = 1011.4098K

Freezing Point (T_b) = 567.4600K

Bioavailabilty Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

D – E5



2-amino -4-oxo -6-phenyl -1-(1-phenylethyl) 1,4,5,6

-tetrahydropyrimidine -5- carbonitrile

Molecular Formula =C19H18N4O

Molecular Mass = 318.37

Hydrogen Bond Acceptor Count = 5

Hydrogen Bond Donor Count = 2

Rotatable Bond Count = 3

Lipophilicity (Log P) = 3

Molar Refractivity = 94.2470cm^3/mol

Liquid Viscosity (η_L) = 0.0014N.s/m^2

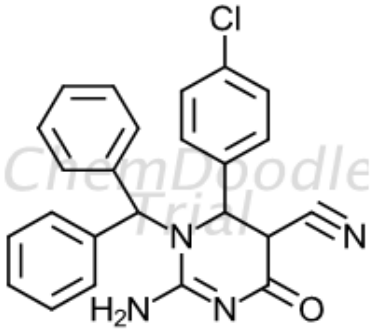
Boiling Point (T_b) = 918.9399K

Freezing Point (T_b) = 581.2800K

Bioavailabilty Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

D – E6



2-amino -6-(4-chloro phenyl) -1-(diphenylmethyl) -4-oxo 1,4,5,6 -tetrahydropyrimidine -5-carbonitrile

Molecular Formula =C24H19ClN4O

Molecular Mass = 414.88

Hydrogen Bond Acceptor Count = 5

Hydrogen Bond Donor Count = 2

Rotatable Bond Count = 5

Lipophilicity (Log P) = 3.44

Molar Refractivity = 119.6350cm^3/mol

Liquid Viscosity (η_L) = 0.0014N.s/m^2

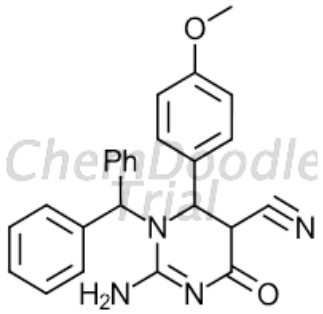
Boiling Point (T_b) = 1097.3298K

Freezing Point (T_b) = 680.0100K

Bioavailabilty Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

D – E7



2-amino -1-(diphenylmethyl) -6-(4-methoxy phenyl) -4-oxo 1,4,5,6 -tetrahydropyrimidine -5-carbonitrile

Molecular Formula =C25H22N4O2

Molecular Mass = 410.46

Hydrogen Bond Acceptor Count = 6

Hydrogen Bond Donor Count = 2

Rotatable Bond Count = 5

Lipophilicity (Log P) = 3.5

Molar Refractivity = 120.8900cm^3/mol

Liquid Viscosity (η_L) = 0.0054N.s/m^2

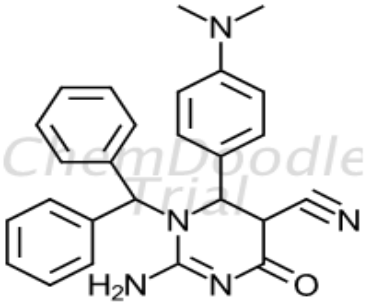
Boiling Point (T_b) = 918.9399K

Freezing Point (T_b) = 581.2800K

Bioavailabilty Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

D – E8



2-amino -6-[4-(dimethyl amino) phenyl] -1-(diphenylmethyl) -4-oxo 1,4,5,6-tetrahydropyrimidine -5-carbonitrile

Molecular Formula =C25H22N4O

Molecular Mass = 425.52

Hydrogen Bond Acceptor Count = 6

Hydrogen Bond Donor Count = 1

Rotatable Bond Count = 5

Lipophilicity (Log P) = 3.6

Molar Refractivity = 127.4301cm^3/mol

Liquid Viscosity (η_L) = 0.0047N.s/m^2

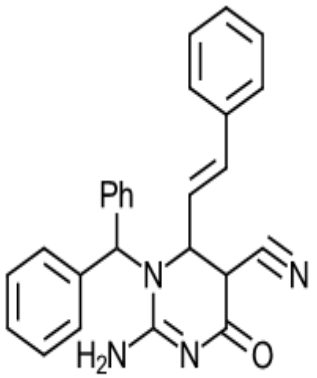
Boiling Point (T_b) = 1144.9298K

Freezing Point (T_b) = 650.9401K

Bioavailabilty Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

D – E9



2-amino -1-(diphenylmethyl) -4-oxo -6-[(E)-2-phenylethenyl]

1,4,5,6-tetrahydropyrimidine -5-carbonitrile

Molecular Formula =C26H22N4O

Molecular Mass = 406.4791

Hydrogen Bond Acceptor Count = 5

Hydrogen Bond Donor Count = 1

Rotatable Bond Count = 5

Lipophilicity (Log P) = 3.77

Molar Refractivity = 124.2490cm^3/mol

Liquid Viscosity (η_L) = 0.0039N.s/m^2

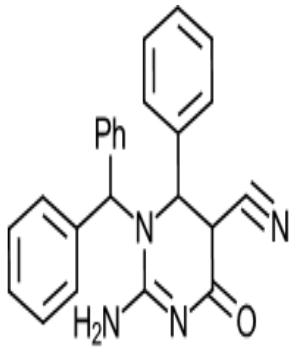
Boiling Point (T_b) = 1136.7699K

Freezing Point (T_b) = 627.3501K

Bioavailabilty Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

D – E10



2-amino -1-(diphenylmethyl) -4-oxo -6-phenyl 1,4,5,6 -tetrahydropyrimidine -5-carbonitrile

Molecular Formula =C24H20N4O

Molecular Mass = 380.44

Hydrogen Bond Acceptor Count = 5

Hydrogen Bond Donor Count = 1

Rotatable Bond Count = 4

Lipophilicity (Log P) = 3.5

Molar Refractivity = 114.4670cm^3/mol

Liquid Viscosity (η_L) = 0.0034N.s/m^2

Boiling Point (T_b) = 1086.8499K

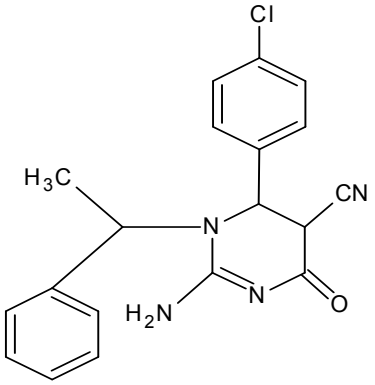
Freezing Point (T_b) = 609.890K

Bioavailabilty Score = 0.5500

Lipinski's Rule of 5 Violations Count

CHEMSKETCH

D – E1

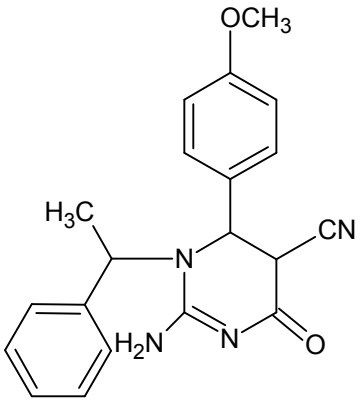


2-amino -6-(4-chloro phenyl)-4-oxo -1-(1-phenylethyl)1,4,5,6

-tetrahydro pyrimidine -5- carbonitrile

Molecular Formula	= $C_{19}H_{17}ClN_4O$
Formula Weight	= 352.81748
Composition	= C(64.68%) H(4.86%) Cl(10.05%) N(15.88%) O(4.53%)
Molar Refractivity	= 98.56 ± 0.5 cm^3
Molar Volume	= 267.6 ± 7.0 cm^3
Parachor	= 718.7 ± 8.0 cm^3
Index of Refraction	= 1.657 ± 0.05
Surface Tension	= 52.0 ± 7.0 dyne/cm
Density	= 1.31 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 39.07 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 352.109089 Da
Nominal Mass	= 352 Da
Average Mass	= 352.8175 Da
M+	= 352.10854 Da
M-	= 352.109637 Da
[M+H]+	= 353.116365 Da
[M+H]-	= 353.117463 Da
[M-H]+	= 351.100715 Da
[M-H]-	= 351.101812 Da

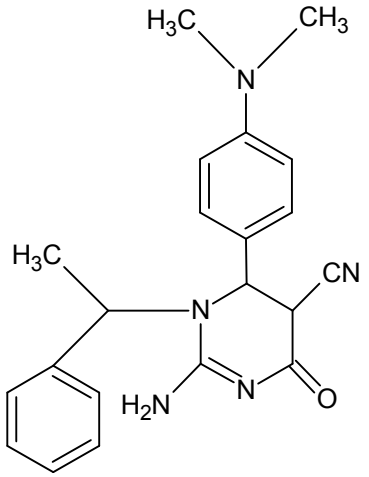
D – E2



2-amin-6-(4-methoxy phenyl) -4-oxo -1- (1-phenylethyl) 1,4,5,6-tetrahydropyrimidine -5-carbonitrile

Molecular Formula	= $C_{20}H_{20}N_4O_2$
Formula Weight	= 348.3984
Composition	= C(68.95%) H(5.79%) N(16.08%) O(9.18%)
Molar Refractivity	= 99.78 ± 0.5 cm^3
Molar Volume	= 280.0 ± 7.0 cm^3
Parachor	= 740.1 ± 8.0 cm^3
Index of Refraction	= 1.631 ± 0.05
Surface Tension	= 48.8 ± 7.0 dyne/cm
Density	= 1.24 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 39.55 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 348.158626 Da
Nominal Mass	= 348 Da
Average Mass	= 348.3984 Da
M+	= 348.158077 Da
M-	= 348.159174 Da
[M+H]+	= 349.165902 Da
[M+H]-	= 349.167 Da
[M-H]+	= 347.150252 Da
[M-H]-	= 347.151349 Da

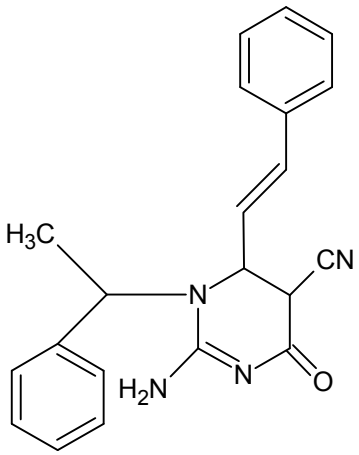
D – E3



2-amino -6-[4-(dimethyl amino) phenyl] -4-oxo -1-(1-phenylethyl) 1,4,5,6-tetrahydropyrimidine -5-carbonitrile

Molecular Formula	= $C_{21}H_{23}N_5O$
Formula Weight	= 361.44022
Composition	= C(69.78%) H(6.41%) N(19.38%) O(4.43%)
Molar Refractivity	= 106.77 ± 0.5 cm^3
Molar Volume	= 299.5 ± 7.0 cm^3
Parachor	= 786.2 ± 8.0 cm^3
Index of Refraction	= 1.631 ± 0.05
Surface Tension	= 47.4 ± 7.0 dyne/cm
Density	= 1.20 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 42.32 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 361.19026 Da
Nominal Mass	= 361 Da
Average Mass	= 361.4402 Da
M+	= 361.189712 Da
M-	= 361.190809 Da
[M+H]+	= 362.197537 Da
[M+H]-	= 362.198634 Da
[M-H]+	= 360.181887 Da
[M-H]-	= 360.182984 Da

D – E4

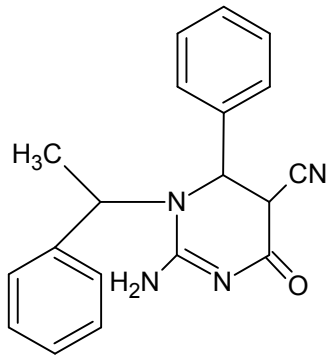


2-amino -4-oxo [(E)-2-phenylethenyl] -1-(1-phenylethyl)

1,4,5,6-tetrahydropyrimidine -5-carbonitrile

Molecular Formula	= $C_{21}H_{20}N_4O$
Formula Weight	= 344.4097
Composition	= C(73.23%) H(5.85%) N(16.27%) O(4.65%)
Molar Refractivity	= 103.18 ± 0.5 cm^3
Molar Volume	= 290.4 ± 7.0 cm^3
Parachor	= 767.1 ± 8.0 cm^3
Index of Refraction	= 1.628 ± 0.05
Surface Tension	= 48.6 ± 7.0 dyne/cm
Density	= 1.18 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 40.90 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 344.163711 Da
Nominal Mass	= 344 Da
Average Mass	= 344.4097 Da
M+	= 344.163163 Da
M-	= 344.16426 Da
[M+H]+	= 345.170988 Da
[M+H]-	= 345.172085 Da
[M-H]+	= 343.155338 Da
[M-H]-	= 343.156435 Da

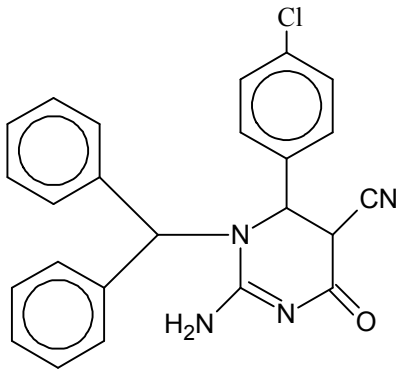
D – E5



2-amino -4-oxo -6-phenyl -1-(1-phenylethyl) 1,4,5,6-tetrahydropyrimidine -5- carbonitrile

Molecular Formula	= $C_{19}H_{18}N_4O$
Formula Weight	= 318.37242
Composition	= C(71.68%) H(5.70%) N(17.60%) O(5.03%)
Molar Refractivity	= 93.96 ± 0.5 cm^3
Molar Volume	= 258.3 ± 7.0 cm^3
Parachor	= 689.9 ± 8.0 cm^3
Index of Refraction	= 1.647 ± 0.05
Surface Tension	= 50.8 ± 7.0 dyne/cm
Density	= 1.23 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 37.25 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 318.148061 Da
Nominal Mass	= 318 Da
Average Mass	= 318.3724 Da
M+	= 318.147513 Da
M-	= 318.14861 Da
[M+H]+	= 319.155338 Da
[M+H]-	= 319.156435 Da
[M-H]+	= 317.139688 Da
[M-H]-	= 317.140785 Da

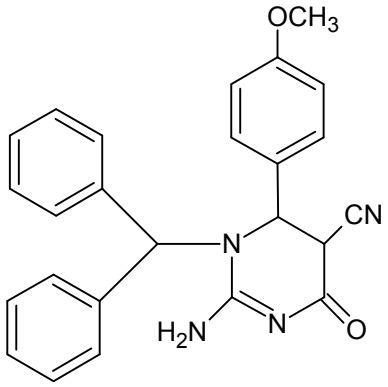
D – E6



2-amino -6-(4-chloro phenyl) -1-(diphenylmethyl) -4-oxo 1,4,5,6-tetrahydropyrimidine -5-carbonitrile

Molecular Formula	= $C_{24}H_{19}ClN_4O$
Formula Weight	= 414.88686
Composition	= C(69.48%) H(4.62%) Cl(8.55%) N(13.50%) O(3.86%)
Molar Refractivity	= 119.25 ± 0.5 cm^3
Molar Volume	= 320.2 ± 7.0 cm^3
Parachor	= 864.5 ± 8.0 cm^3
Index of Refraction	= 1.667 ± 0.05
Surface Tension	= 53.0 ± 7.0 dyne/cm
Density	= 1.29 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 47.27 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 414.124739 Da
Nominal Mass	= 414 Da
Average Mass	= 414.8869 Da
M+	= 414.12419 Da
M-	= 414.125288 Da
[M+H]+	= 415.132015 Da
[M+H]-	= 415.133113 Da
[M-H]+	= 413.116365 Da
[M-H]-	= 413.117463 Da

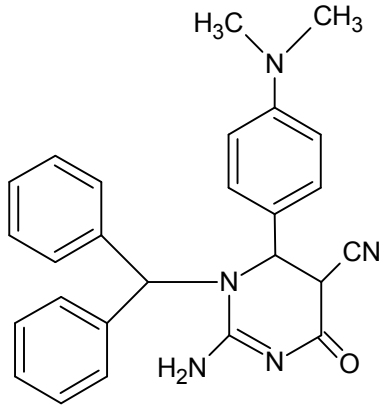
D – E7



2-amino -1-(diphenylmethyl) -6-(4-methoxy phenyl) -4-oxo 1,4,5,6-tetrahydropyrimidine -5-carbonitrile

Molecular Formula	= $C_{25}H_{22}N_4O_2$
Formula Weight	= 410.46778
Composition	= C(73.15%) H(5.40%) N(13.65%) O(7.80%)
Molar Refractivity	= 120.46 ± 0.5 cm^3
Molar Volume	= 332.6 ± 7.0 cm^3
Parachor	= 885.9 ± 8.0 cm^3
Index of Refraction	= 1.644 ± 0.05
Surface Tension	= 50.3 ± 7.0 dyne/cm
Density	= 1.23 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 47.75 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 410.174276 Da
Nominal Mass	= 410 Da
Average Mass	= 410.4678 Da
M+	= 410.173727 Da
M-	= 410.174825 Da
[M+H]+	= 411.181552 Da
[M+H]-	= 411.18265 Da
[M-H]+	= 409.165902 Da
[M-H]-	= 409.167 Da

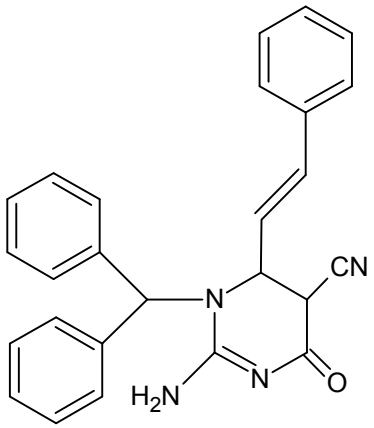
D – E8



2-amino -6-[4-(dimethyl amino) phenyl] -1-(diphenylmethyl) -4-oxo 1,4,5,6-tetrahydropyrimidine -5-carbonitrile

Molecular Formula	= $C_{26}H_{25}N_5O$
Formula Weight	= 425.5096
Composition	= C(73.74%) H(5.95%) N(16.54%) O(3.78%)
Molar Refractivity	= 127.45 ± 0.5 cm^3
Molar Volume	= 352.1 ± 7.0 cm^3
Parachor	= 932.0 ± 8.0 cm^3
Index of Refraction	= 1.643 ± 0.05
Surface Tension	= 49.0 ± 7.0 dyne/cm
Density	= 1.20 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 50.52 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 423.20591 Da
Nominal Mass	= 423 Da
Average Mass	= 423.5096 Da
M+	= 423.205362 Da
M-	= 423.206459 Da
[M+H]+	= 424.213187 Da
[M+H]-	= 424.214284 Da
[M-H]+	= 422.197537 Da
[M-H]-	= 422.198634 Da

D – E9

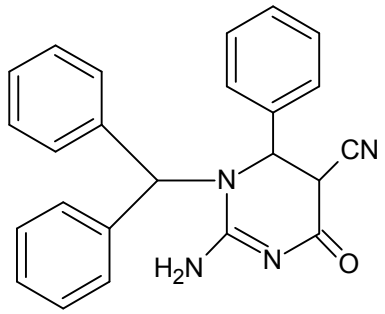


2-amino -1-(diphenylmethyl) -4-oxo -6-[(E)-2-phenylethenyl]

1,4,5,6-tetrahydropyrimidine -5-carbonitrile

Molecular Formula	= $C_{26}H_{22}N_4O$
Formula Weight	= 406.47908
Composition	= C(76.83%) H(5.46%) N(13.78%) O(3.94%)
Molar Refractivity	= 123.86 ± 0.5 cm^3
Molar Volume	= 343.1 ± 7.0 cm^3
Parachor	= 912.9 ± 8.0 cm^3
Index of Refraction	= 1.641 ± 0.05
Surface Tension	= 50.1 ± 7.0 dyne/cm
Density	= 1.18 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 49.10 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 406.179361 Da
Nominal Mass	= 406 Da
Average Mass	= 406.4791 Da
M+	= 406.178813 Da
M-	= 406.17991 Da
[M+H]+	= 407.186638 Da
[M+H]-	= 407.187735 Da
[M-H]+	= 405.170988 Da
[M-H]-	= 405.172085 Da

D – E10



2-amino -1-(diphenylmethyl) -4-oxo -6-phenyl 1,4,5,6

-tetrahydropyrimidine -5-carbonitrile

Molecular Formula	= $C_{24}H_{20}N_4O$
Formula Weight	= 380.4418
Composition	= C(75.77%) H(5.30%) N(14.73%) O(4.21%)
Molar Refractivity	= 114.65 ± 0.5 cm^3
Molar Volume	= 311.0 ± 7.0 cm^3
Parachor	= 835.6 ± 8.0 cm^3
Index of Refraction	= 1.658 ± 0.05
Surface Tension	= 52.1 ± 7.0 dyne/cm
Density	= 1.22 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 45.45 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 380.163711 Da
Nominal Mass	= 380 Da
Average Mass	= 380.4418 Da
M+	= 380.163163 Da
M-	= 380.16426 Da
[M+H]+	= 381.170988 Da
[M+H]-	= 381.172085 Da
[M-H]+	= 379.155338 Da
[M-H]-	= 379.156435 Da

LIPINSKI'S RULE OF THE SYNTHESIZED COMPOUND (Table No: 1)

COMPOUND CODE	MOLECULAR WEIGHT	LOG P	H-BOND DONOR	H-BOND ACCEPTOR	MR	NO OF CRETERIA
RULE	<500	<5	<5	<10	40-130cm^3/mol	ATLEAST 3
D – E1	352.81	2.89	1	5	98.9070	ALL
D – E2	348.90	3	1	6	100.1620	ALL
D – E3	361.44	3.3	1	6	106.9560	ALL
D –E4	344.41	3.22	1	5	103.7560	ALL
D – E5	318.37	3	2	5	94.2470	ALL
D – E6	414.88	3.44	2	5	119.6350	ALL
D – E7	410.46	3.5	1	6	120.89	ALL
D – E8	425.52	3.6	1	6	127.430	ALL
D – E9	406.47	3.77	1	5	124.2490	ALL
D – E10	380.44	3.5	1	5	114.4670	ALL

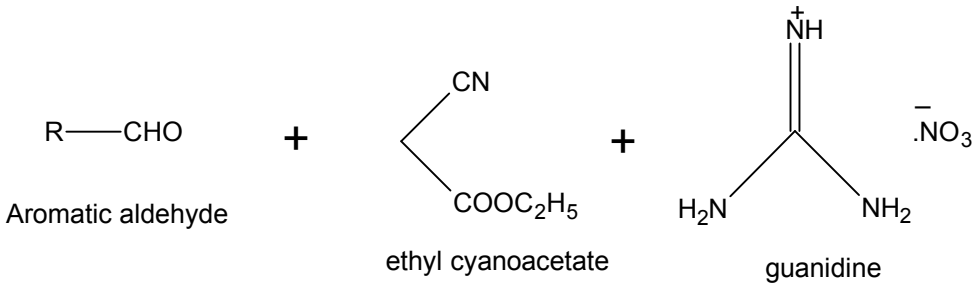
EXPERIMENTAL PROCEDURE

5. EXPERIMENTAL PROCEDURE

SCHEME OF REACTION

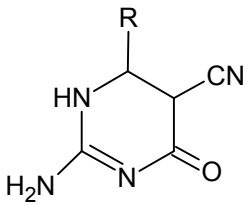
STEP 1:

PREPARATION OF 2-AMINO-4-OXO -6-ARYL 1,4,5,6–TETROHYDROPYRIMIDINE -5-CARBONITRILE



K_2CO_3

H_2O,reflux at 70 - 80 temp



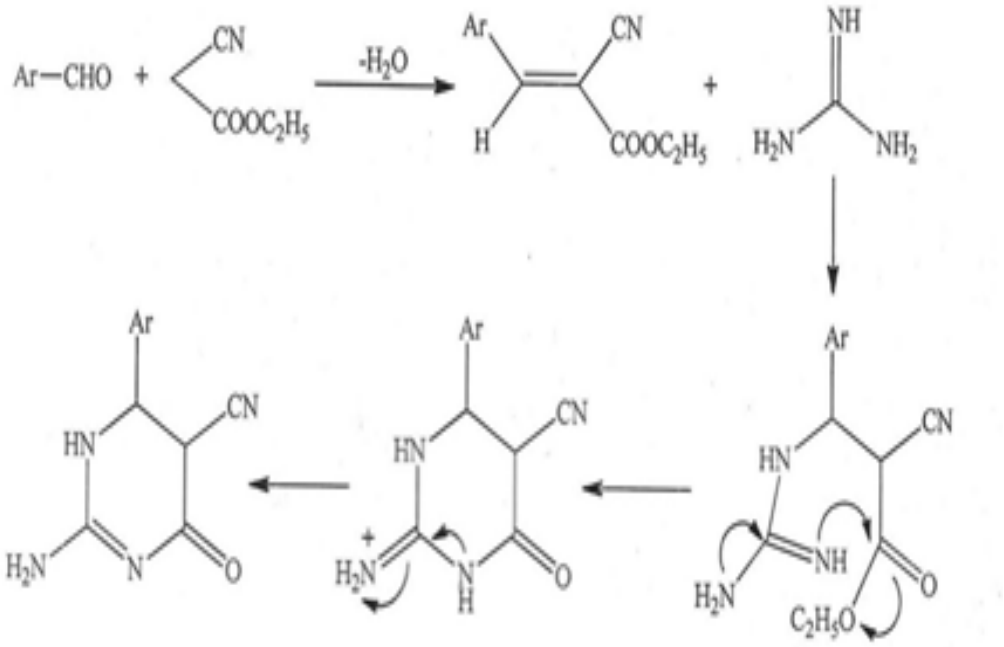
2-amino 6_ aryl_4_ oxo 1,4,5,6_ tetrahydropyrimidine 5_ carbonitrile

R ⟹

1. P-Chloro benzaldehyde
2. P-Methoxy benzaldehyde
3. p-Dimethylamino benzaldehyde
4. Cinnamaldehyde
5. Bezaldehyde

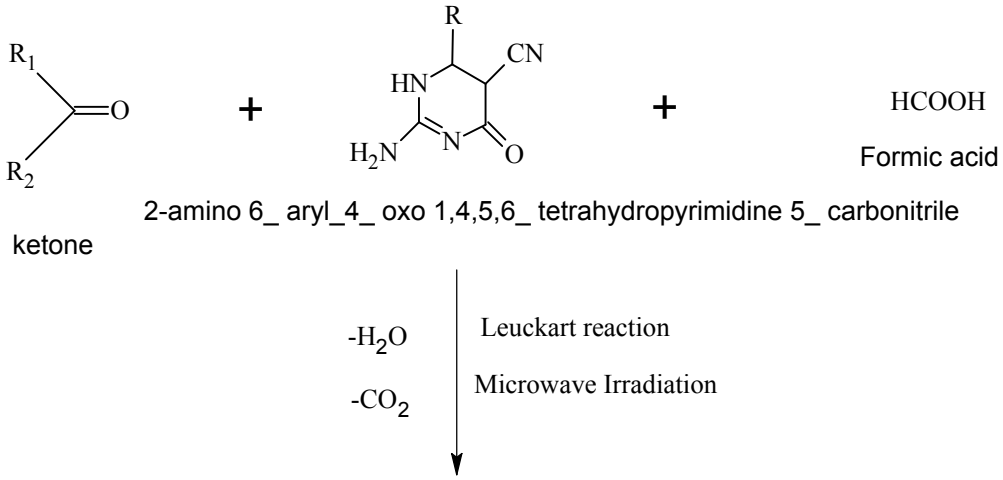
Mechanism:

- Aromatic aldehyde reacts with ethyl cyanoacetate to form arylmethylene ethyl cyanoactate.
- Subsequently added with quanidine fallowed by cyclization and tautomarisation to form 2-amino-4-oxo-6-aryl 1, 4, 5, 6-tetrahydropyrimidine-5-carbonitrile.



STEP 2:

PREPARATION OF 1-SUBSTITUTED TETRAHYDROPYRIMIDINE DERIVATIVES FROM 2-AMINO-4-OXO - 6-ARYL 1, 4, 5, 6 TETRAHYDROPYRIMIDINE -5-CARBONITRILE.



ketone

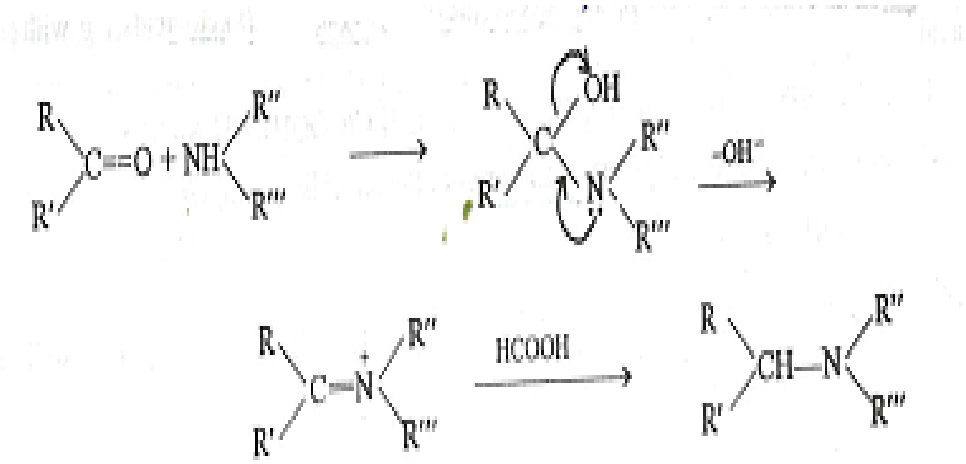
2-amino 6_ aryl_4_ oxo 1,4,5,6_ tetrahydropyrimidine 5_ carbonitrile

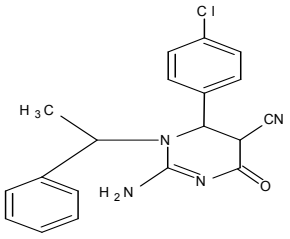
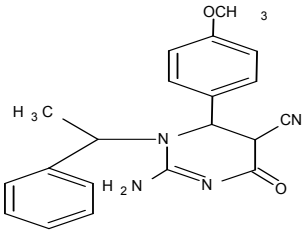
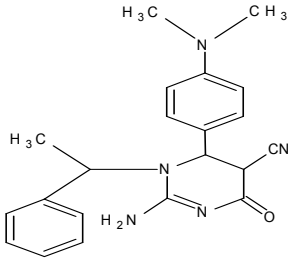
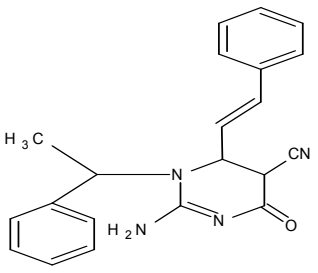
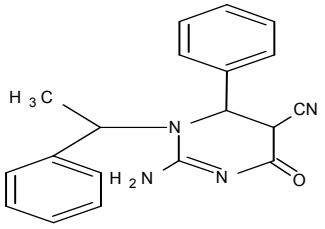
1_substituted 2-amino 6_ aryl_4_ oxo 1,4,5,6_ tetrahydropyrimidine 5_ carbonitrile

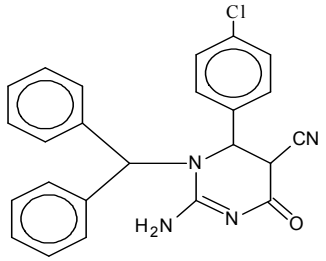
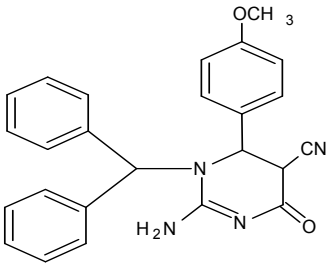
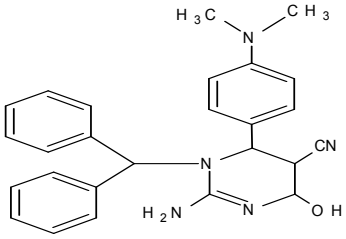
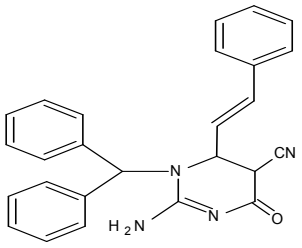
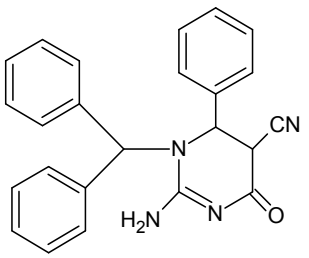
COMPOUND	R1	R2
D –E1 to D –E5	CH3	C6H5
D –E6 to D- E10	C6H5	C6H5

Mechanism

- ✓ Amine reacst with protonated ketone to give iminium ion.
- ✓ The iminium ion then react with formic acid to give methylated ammonium ion and release CO_2 gas, where formic acid act as a reducing agent or hydride transfer reagent.
- ✓ This CO_2 gas leads the synthesis process to the next level of synthesis.
- ✓ In this stage ammonium ion gets deprotonated to form final methylated amine product.
- ✓ If reaction occurs with primary amine same process follows twice to reach the tertiary amine as a final product.



COMPOUND CODE	SYNTHESIZED COMPOUND
D – E1	
D – E2	
D – E3	
D – E4	
D – E5	

D – E6	
D – E7	
D – E8	
D – E9	
D – E10	

EXPERIMENTAL PROCEDURE

COMPOUND –A

Synthesis of 2-amino -4-oxo- 6-(4-choloro phenyl) 1, 4, 5, 6-tetrahydropyrimidine -5-carbonitrile

CHEMICALS REQUIRED

- Guanidine nitrate -1.5mmol
- Ethyl cyanoacetate -1.2mmo
- P-Chloro benzaldehyde -1.0mmol
- Potassium carbonate -Q.S

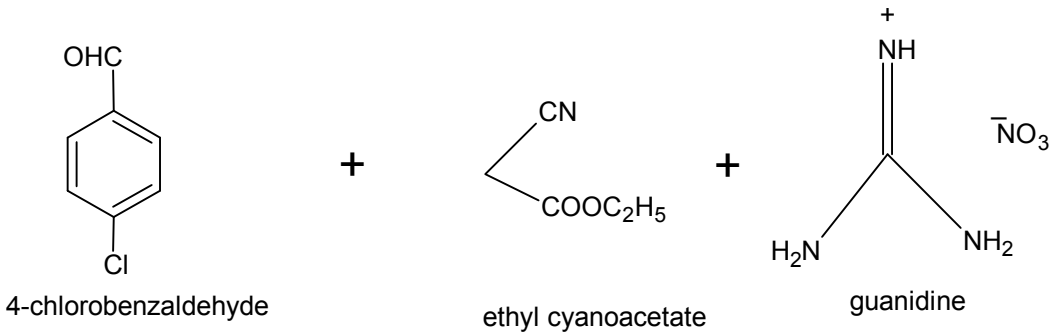
PROCEDURE

A mixture of p-chloro benzaldehyde (1mmol), guanidine nitrate (1.5mmol), ethyl cyanoacetate (1.2mmol) and catalytic amount of potassium carbonate was taken in a round bottom flask (100ml) with water as a solvent and refluxed at 100◦C. The progress of the reaction was monitored by TLC. The solution was poured into ice cold water. After the completion of the reaction the solid product was collected by filteration. The product was dried and recrystallized from hot ethanol to obtained pure product.

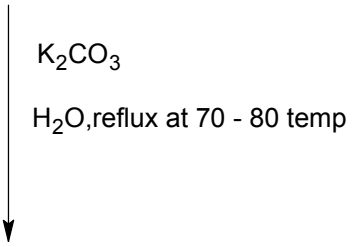
PREPARATION OF 2-AMINO-4-OXO-6-(4-CHLORO PHENYL) 1, 4, 5, 6-

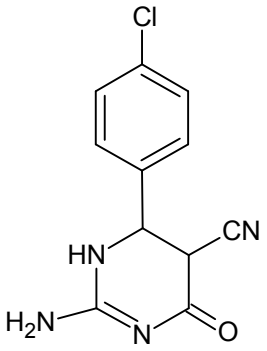
TETRAHYDROPYRIMIDINE -5-CARBONITRILE

COMPOUND - A



4-chlorobenzaldehyde ethyl cyanoacetate guanidine





2-amino-6-(4-chlorophenyl)-4-oxo-1,4,5,6-tetrahydropyrimidine-5-carbonitrile

COMPOUND –B

Synthesis of 2-amino -4-oxo -6-(4-methoxy phenyl) 1, 4, 5, 6-tetrahydropyrimidine -5-carbonitrile

CHEMICALS REQUIRED

- Guanidine nitrate -1.5mmol
- Ethyl cyanoacetate -1.2mmol
- P-Methoxy benzaldehyde -1.0mmol
- Potassium carbonate -Q.S

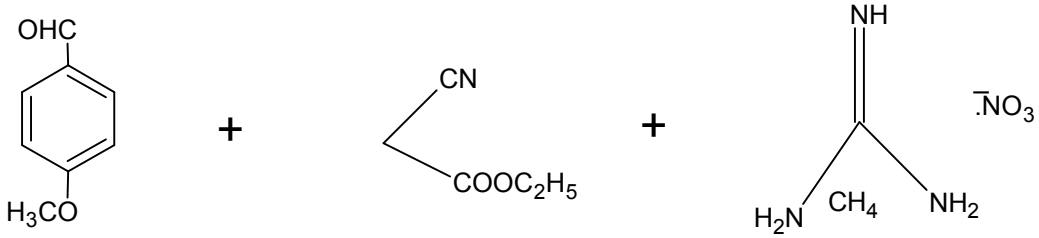
PROCEDURE

A mixture of p-methoxy benzaldehyde (1mmol), guanidine nitrate (1.5mmol), ethyl cyanoacetate (1.2mmol) and catalytic amount of potassium carbonate was taken in a round bottom flask (100ml) with water as a solvent and refluxed at 100◦C. The progress of the reaction was monitored by TLC. The solution was poured into ice cold water. After the completion of the reaction the solid product was collected by filteration. The product was dried and recrystallized from hot ethanol to obtained pure product.

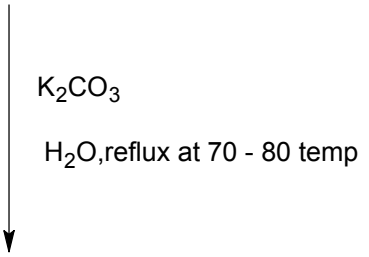
PREPARATION OF 2- AMINO – 6-(4-METHOXY PHENYL) 1, 4, 5, 6-

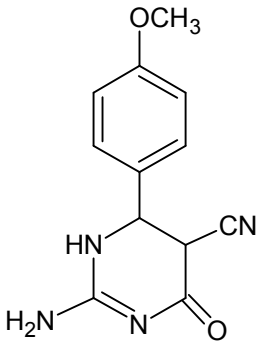
TETRAHYDROPYRIMIDINE -5-CARBONITRILE

COMPOUND - B



4-methoxybenzaldehyde ethyl cyanoacetate guanidine





2-amino-6-(4-methoxyphenyl)-4-oxo-1,4,5,6-tetrahydropyrimidine-5-carbonitrile

COMPOUND –C

Synthesis of 2-amino6-[4-(dimethylamino) phenyl]-4-oxo 1, 4, 5, 6 - tetrahydropyrimidine -5-carbonitrile

CHEMICALS REQUIRED

- Guanidine nitrate -1.5mmol
- Ethyl cyanoacetate -1.2mmol
- P-Dimethylamino benzaldehyde -1.0mmol
- Potassium carbonate -Q.S

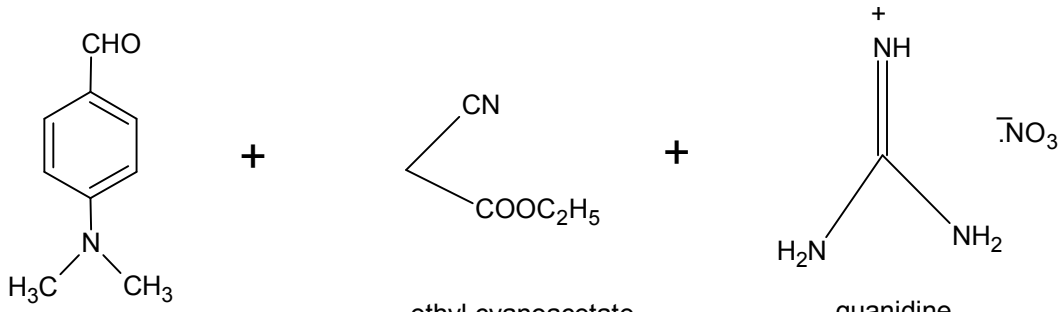
PROCEDURE

A mixture of p-dimethylamino benzaldehyde (1mmol), guanidine nitrate (1.5mmol), ethyl cyanoacetate (1.2mmol) and catalytic amount of potassium carbonate was taken in a round bottom flask (100ml) with water as a solvent and refluxed at 100◦C.The progress of the reaction was monitored by TLC. The solution was poured into ice cold water. After the completion of the reaction the solid product was collected by filteration. The product was dried and recrystallized from hot ethanol to obtained pure product.

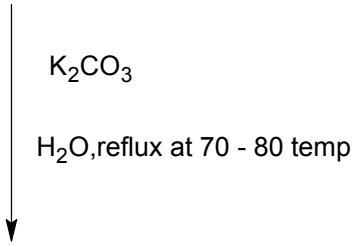
PREPARATION 2-AMINO-6-[4-(DIMETHYLAMINO) PHENY] -4-OXO 1, 4, 5, 6-

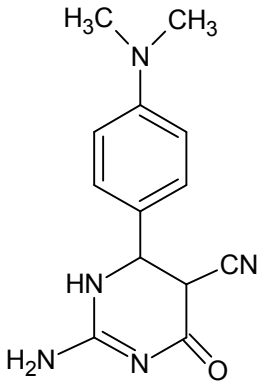
TETRAHYDROPYRIMIDINE-5-CARBONITRILE

COMPOUND - C



4-(dimethylamino)benzaldehyde ethyl cyanoacetate guanidine





2-amino-6-[4-(dimethylamino)phenyl]-4-oxo-1,4,5,6-tetrahydropyrimidine-5-carbonitrile

COMPOUND-D

Synthesis of 2-amino-4-oxo-6-[(E)-2 phenyl ethenyl] 1, 4, 5, 6-tetrahydropyrimidine -5-carbonitrile

CHEMICALS REQUIRED

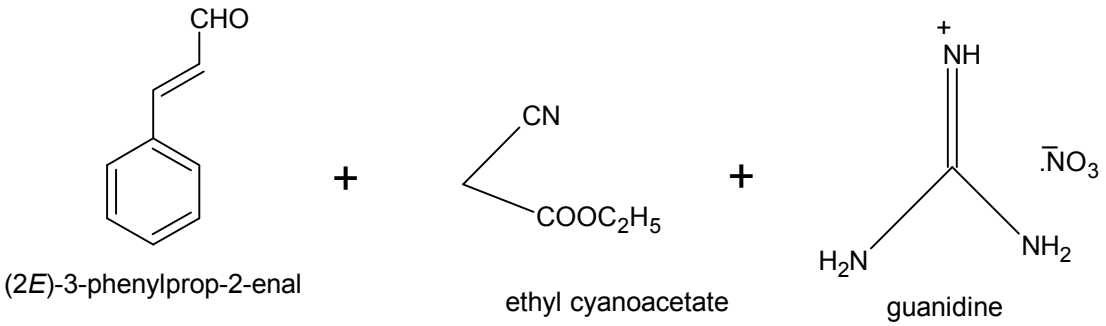
- Guanidine nitrate -1.5mmol
- Ethyl cyanoacetate -1.2mmol
- Cinnamaldehyde -1.0mmol -1.0mmol
- Potassium carbonate -Q.S

PROCEDURE

A mixture of cinnamaldehyde (1.0mmol), guanidine nitrate (1.5mmol), ethyl cyanoacetate (1.2mmol) and catalytic amount of potassium carbonate was taken in a round bottom flask (100ml) with water as a solvent and refluxed at 100◦C. The progress of the reaction was monitored by TLC. The solution was poured into ice cold water. After the completion of the reaction the solid product was collected by filteration. The product was dried and recrystallized from hot ethanol to obtained pure product.

PREPARATION OF 2-AMINO-4-OXO -6-[(E)-2-PHENYL ETHENYL] 1, 4, 5, 6-TETRAHYDROPYRIMIDINE-5-CARBONITRILE

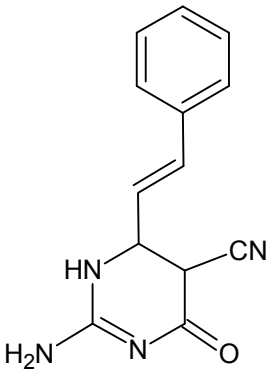
COMPOUND - D



(2*E*)-3-phenylprop-2-enal + ethyl cyanoacetate + guanidine

K_2CO_3

H_2O,reflux at 70 - 80 temp



2-amino-4-oxo-6-[(*E*)-2-phenylethenyl]-1,4,5,6-tetrahydropyrimidine-5-carbonitrile

COMPOUND-E

Synthesis 0f 2-amino -4-oxo -6-phenyl 1, 4, 5, 6-tetrahydropyrimidine -5-carbonitrile

CHEMICALS REQUIRED

- Guanidine nitrate -1.5mmol
- Ethyl cyanoacetate -1.2mmol
- Benzaldehyde -1.0mmol
- Potassium carbonate -Q.S

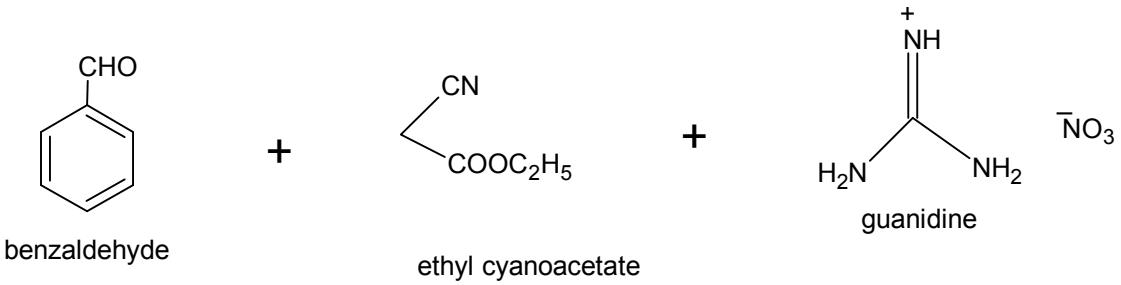
PROCEDURE

A mixture of benzaldehyde (1.0mmol), guanidine nitrate (1.5mmol), ethyl cyanoacetate (1.2mmol) and catalytic amount of potassium carbonate was taken in a round bottom flask (100ml) with water as a solvent and refluxed at 100◦C. The progress of the reaction was monitored by TLC. The solution was poured into ice cold water. After the completion of the reaction the solid product was collected by filteration. The product was dried and recrystallized from hot ethanol to obtained pure product.

PREPARATION OF 2-AMINO-4-OXO -6-PHENYL 1, 4, 5, 6 -

TETRAHYDRO PYRIMIDINE -5-CARBONITRILE

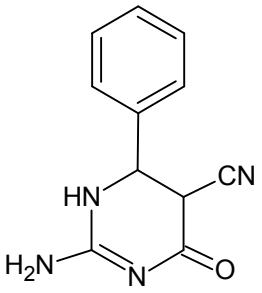
COMPOUND -E



benzaldehyde ethyl cyanoacetate guanidine

K_2CO_3

H_2O,reflux at 70 - 80 temp



2-amino-4-oxo-6-phenyl-1,4,5,6-tetrahydropyrimidine-5-carbonitrile

COMPOUND DERIVATIVES

COMPOUND D –E1

CHEMICALS REQUIRED

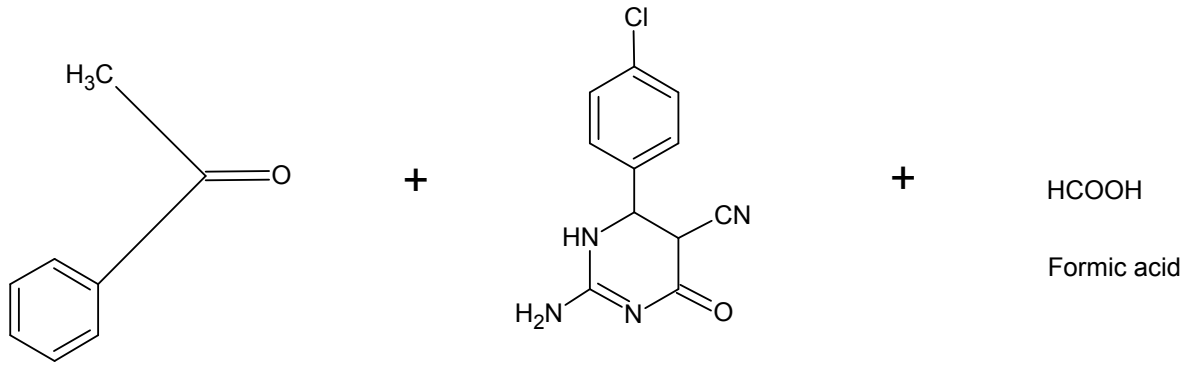
- Acetophenone -1.5ml
- 2-amino -6-(4-chloro phenyl) -4-oxo

  1, 4, 5, 6-tetrahydroPyrimidine -5-carbonitrile -1.5gm
- Formic acid -1.5ml

PROCEDURE

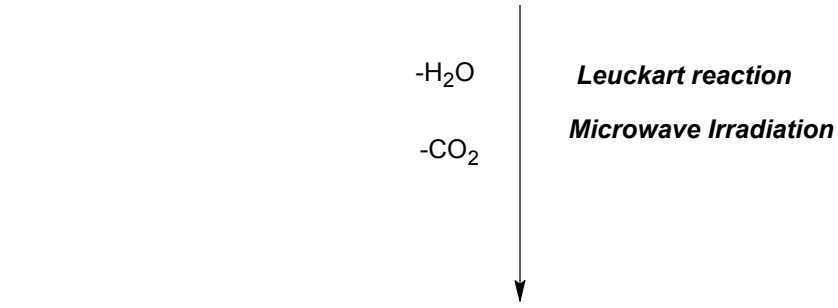
Acetophenone (1.5ml), 2-amino -6-(4-chloro phenyl) -4-oxo 1,4,5,6-tetrahydropyrimidine -5-carbonitrile (1.5gm) and formic acid (1.5ml) was irradiated in microwave at 80◦C for 4minutes in equimolar quantities. The solution was obtained and then cooled in ice bath until the crystals are formed. The crude product was obtained and washed with ice cold water and air dried.

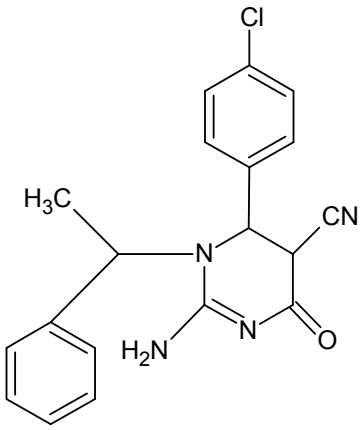
COMPOUND D - E_1



1-phenylethanone

2-amino-6-(4-chlorophenyl)-4-oxo-1,4,5,6-tetrahydropyrimidine-5-carbonitrile





2-amino-6-(4-chlorophenyl)-4-oxo-1-(1-phenylethyl)-1,4,5,6-tetrahydropyrimidine-5-carbonitrile

COMPOUND D – E2

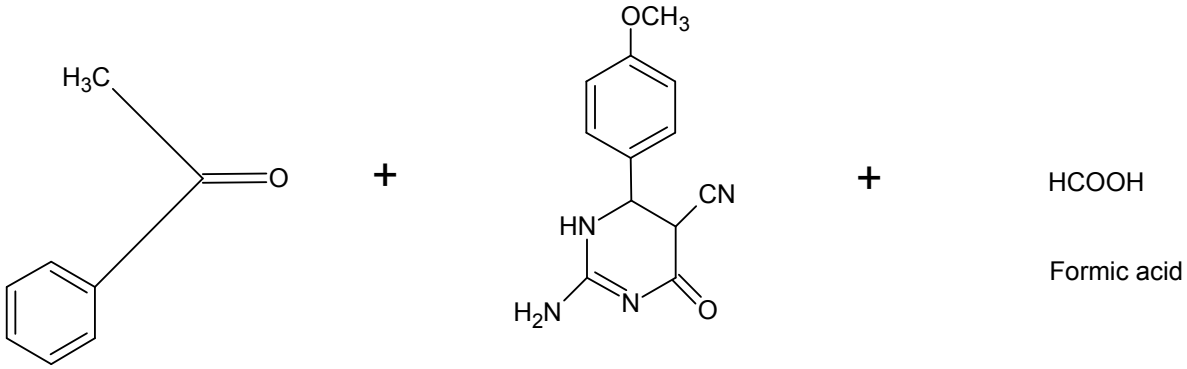
CHEMICALS REQUIRED

- Acetophenone -1.5ml
- 2-amino -6-(4-methoxy phenyl)- 4- oxo
  1, 4, 5, 6-tetrahydropyrimidine -5-carbonitrile -1.5gm
- Formic acid -1.5ml

PROCEDURE

Acetophenone (1.5ml),2-amino -6-(4-methoxy phenyl) -4-oxo 1,4,5,6-tetrahydropyrimidine -5-carbonitrile(1.5gm) and formic acid(1.5ml) was irradiated in microwave at 80◦C for 4minutes in equimolar quantities. The solution was obtained and then cooled in ice bath until the crystals are formed. The crude product was obtained and washed with ice cold water and air dried.

COMPOUND D - E_2



1-phenylethanone

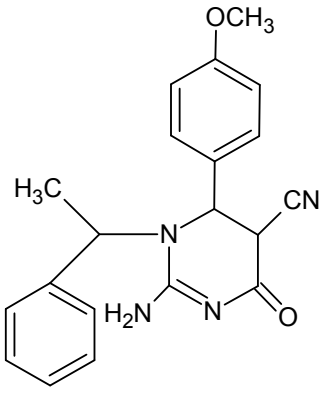
2-amino-6-(4-methoxyphenyl)-4-oxo-1,4,5,6-tetrahydropyrimidine-5-carbonitrile

$-H_2O$

$-CO_2$

Leuckart reaction

Microwave Irradiation



2-amino-6-(4-methoxyphenyl)-4-oxo-1-(1-phenylethyl)-1,4,5,6-tetrahydropyrimidine-5-carbonitrile

COMPOUND D – E3

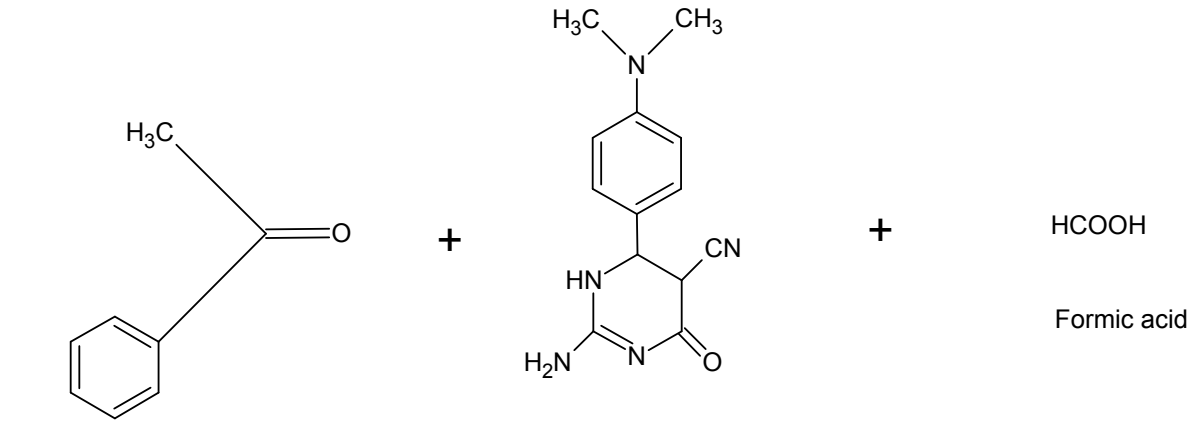
CHEMICALS REQUIRED

- Acetophenone -1.5ml
- 2-amino -6-(4-dimethyl amino phenyl)- 4- oxo 1, 4, 5, 6-tetrahydropyrimidine -5-carbonitrile -1.5gm
- Formic acid -1.5ml

PROCEDURE:

Acetophenon (1.5ml),2-amino -6-(4-dimethyl amino phenyl) -4-oxo 1,4,5,6 -tetrahydropyrimidine -5-carbonitrile(1.5gm) and formic acid(1.5ml) was irradiated in microwave at 80◦C for 4minutes in equimolar quantities. The solution was obtained and then cooled in ice bath until the crystals are formed. The crude product was obtained and washed with ice cold water and air dried.

COMPOUND D - E_3



1-phenylethanone

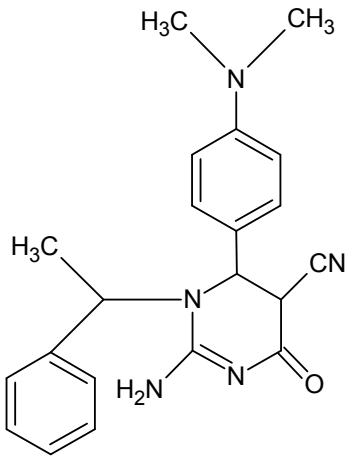
2-amino-6-[4-(dimethylamino)phenyl]-4-oxo-1,4,5,6-tetrahydropyrimidine-5-carbonitrile

$-H_2O$

$-CO_2$

Leuckart reaction

Microwave Irradiation



2-amino-6-[4-(dimethylamino)phenyl]-4-oxo-1-(1-phenylethyl)-1,4,5,6-tetrahydropyrimidine-5-carbonitrile

COMPOUND D – E4

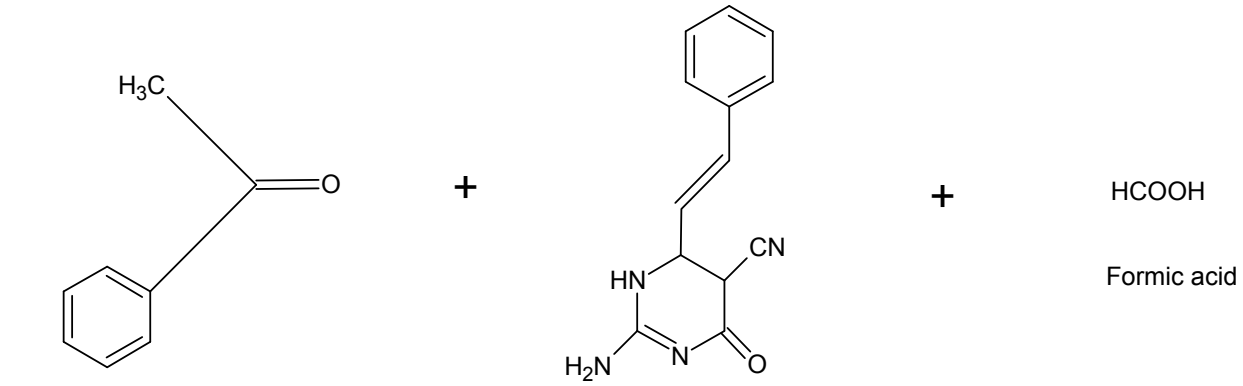
CHEMICALS REQUIRED

- Acetophenone -1.5ml
- 2-amino - 4- oxo -6-[(E)-2-phenyl ethenyl] 1, 4, 5, 6-tetrahydropyrimidine -5-carbonitrile -1.5gm
- Formic acid -1.5ml

PROCEDURE

Acetophenone (1.5ml),2-amino -4-oxo -6-[(E)-2-phenyl ethenyl] 1,4,5,6-tetrahydropyrimidine -5-carbonitrile(1.5gm) and formic acid(1.5ml) was irradiated in microwave at 80◦C for 4minutes in equimolar quantities. The solution was obtained and then cooled in ice bath until the crystals are formed. The crude product was obtained and washed with ice cold water and air dried.

COMPOUND D - E_4

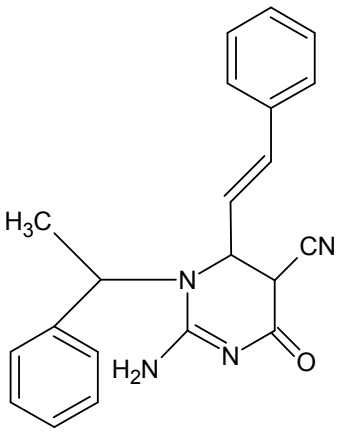


1-phenylethanone

2-amino-4-oxo-6-[(*E*)-2-phenylethenyl]-1,4,5,6-tetrahydropyrimidine-5-carbonitrile

$-H_2O$ ***Leuckart reaction***

$-CO_2$ ***Microwave Irradiation***



2-amino-4-oxo-6-[(*E*)-2-phenylethenyl]-1-(1-phenylethyl)-1,4,5,6-tetrahydropyrimidine-5-carbonitrile

COMPOUND D –E5

CHEMICALS REQUIRED

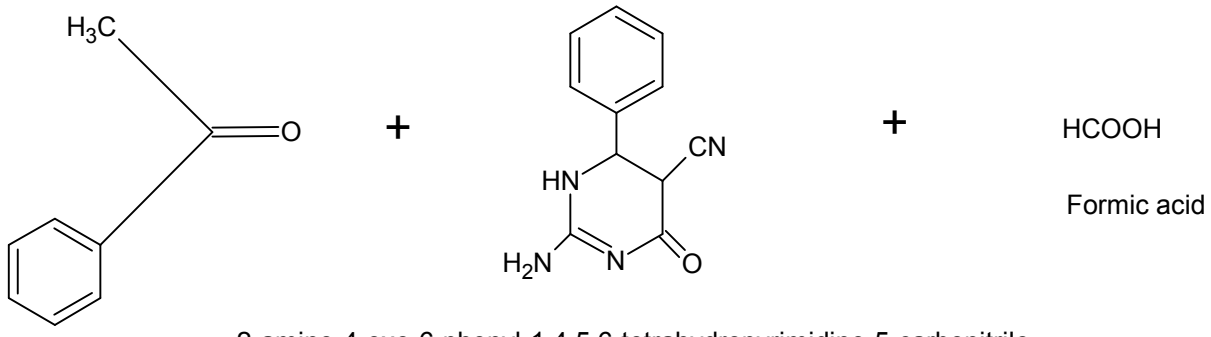
- Acetophenone -1.5ml
- 2-amino - 4- oxo -6-phenyl

  1, 4, 5, 6-tetrahydropyrimidine -5-carbonitrile -1.5gm
- Formic acid -1.5ml

PROCEDURE

Acetophenone (1.5ml), 2-amino -4-oxo -6-phenyl 1,4,5,6-tetrahydropyrimidine -5-carbonitrile(1.5gm) and formic acid(1.5ml) was irradiated in microwave at 80◦C for 4minutes in equimolar quantities. The solution was obtained and then cooled in ice bath until the crystals are formed. The crude product was obtained and washed with ice cold water and air dried.

COMPOUND D - E_5

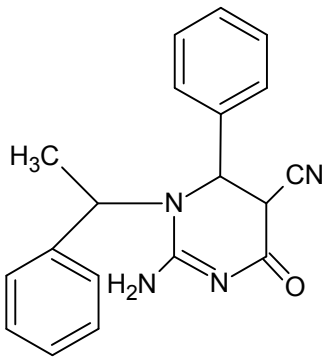


1-phenylethanone

2-amino-4-oxo-6-phenyl-1,4,5,6-tetrahydropyrimidine-5-carbonitrile

$-H_2O$ ***Leuckart reaction***

$-CO_2$ ***Microwave Irradiation***



2-amino-4-oxo-6-phenyl-1-(1-phenylethyl)-1,4,5,6-tetrahydropyrimidine-5-carbonitrile

COMPOUND D –E6

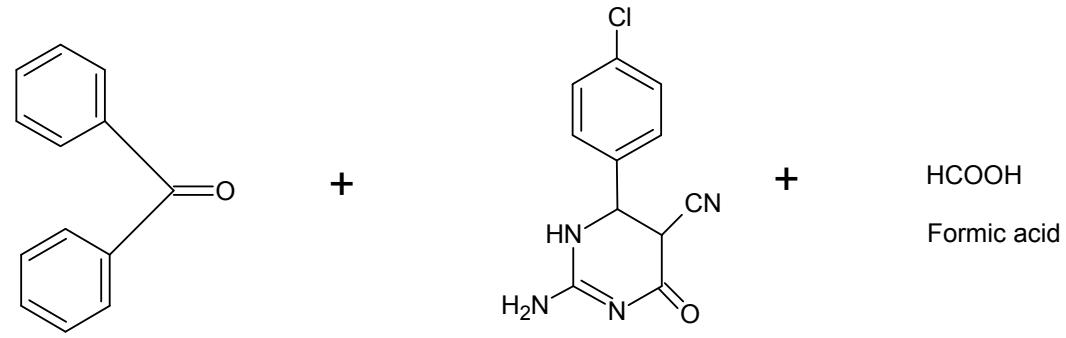
CHEMICALS REQUIRED

- Benzophenone -1.5ml
- 2-amino -6-(4-chloro phenyl) -4-oxo
  1, 4, 5, 6-tetrahydropyrimidine -5-carbonitrile -1.5gm
- Formic acid -1.5ml

PROCEDURE

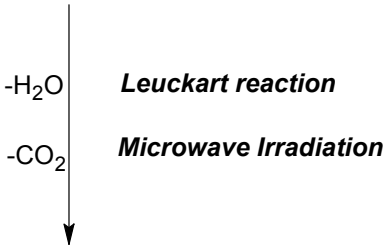
Benzophenone (1.5ml), 2-amino -6-(4-chloro phenyl) 1,4,5,6-tetrahydropyrimidine -5-carbonitrile (1.5gm) and formic acid (1.5ml) was irradiated in microwave at 80◦C for 4minutes in equimolar quantities. The solution was obtained and then cooled in ice bath until the crystals are formed. The crude product was obtained and washed with ice cold water and air dried.

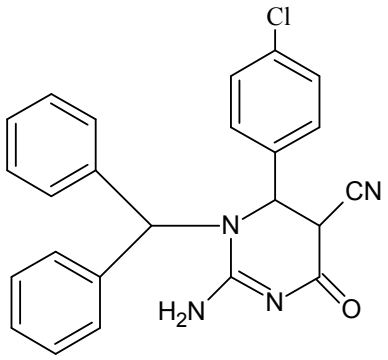
COMPOUND D - E_6



diphenylmethanone

2-amino-6-(4-chlorophenyl)-4-oxo-1,4,5,6-tetrahydropyrimidine-5-carbonitrile





2-amino-6-(4-chlorophenyl)-1-(diphenylmethyl)-4-oxo-1,4,5,6-tetrahydropyrimidine-5-carbonitrile

COMPOUND D –E7

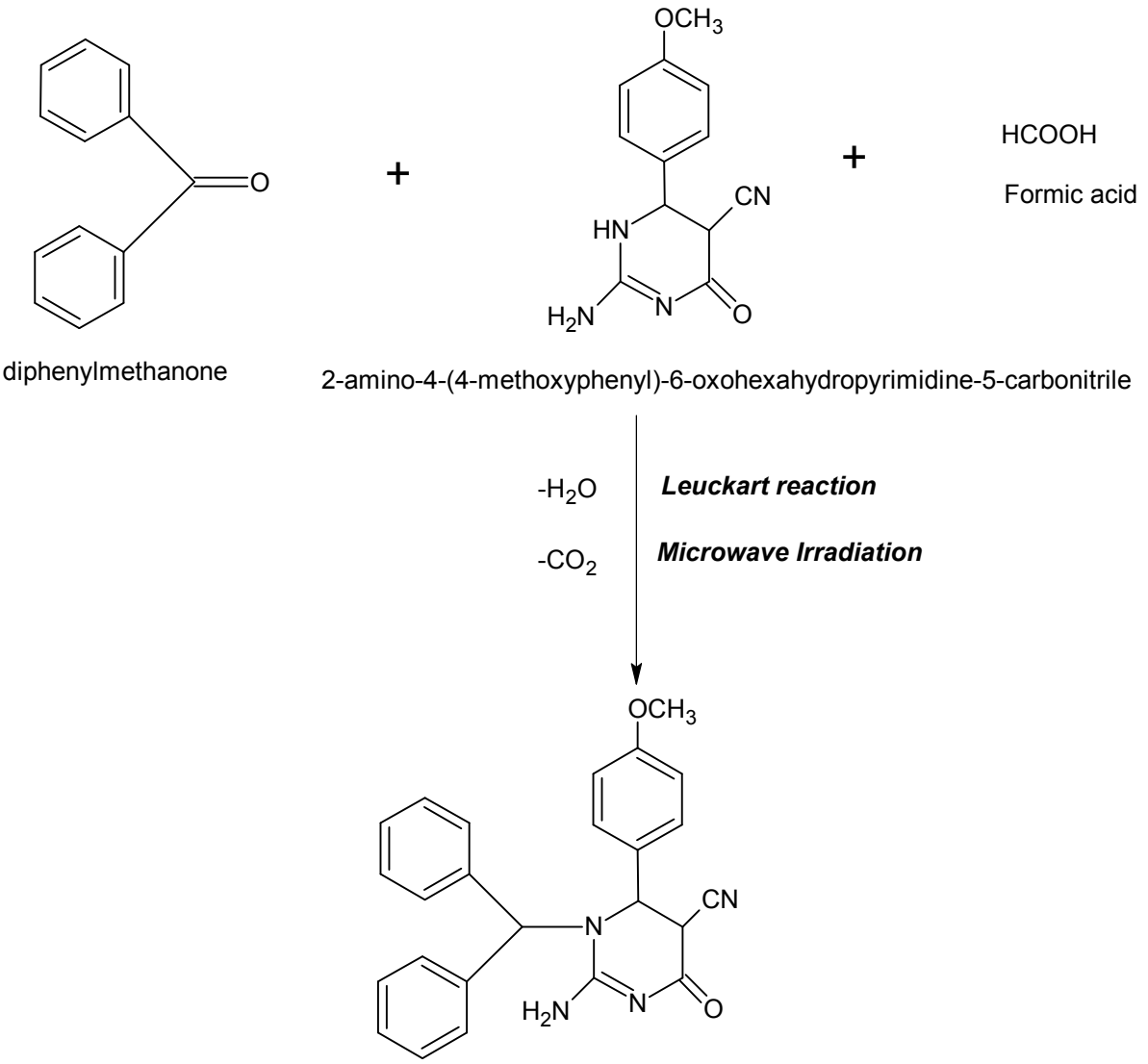
CHEMICALS REQUIRED

- Benzophenone -1.5ml
- 2-amino -6-(4-methoxy phenyl) -4-oxo
  1, 4, 5, 6-tetrahydropyrimidine -5-carbonitrile -1.5gm
- Formic acid -1.5ml

PROCEDURE:

Benzophenone (1.5ml), 2-amino -6-(4-methoxy phenyl) 1,4,5,6-tetrahydropyrimidine -5-carbonitrile (1.5gm) and formic acid(1.5ml) was irradiated in microwave at 80◦C for 4minutes in equimolar quantities. The solution was obtained and then cooled in ice bath until the crystals are formed. The crude product was obtained and washed with ice cold water and air dried.

COMPOUND D - E_7



2-amino -1-(diphenyl methyl) 6-(4-methoxy phenyl) -4-oxo 1,4,5,6-tetrahydropyrimidine-5-carbonitrile

COMPOUND D –E8

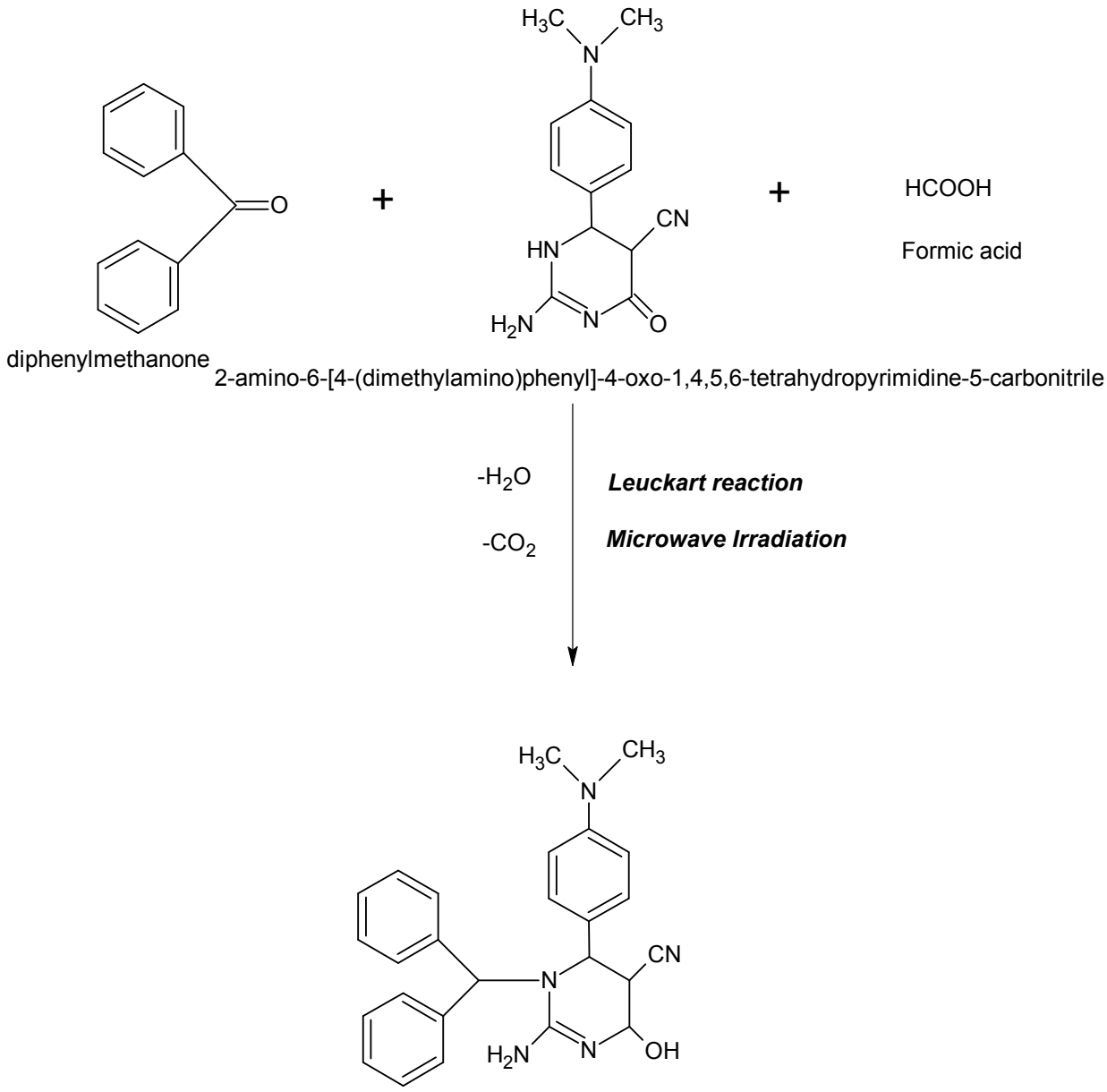
CHEMICALS REQUIRED

- Benzophenone -1.5ml
- 2-amino -6-(4-dimethyl amino phenyl)- 4- oxo 1, 4, 5, 6-tetrahydropyrimidine -5-carbonitrile -1.5gm
- Formic acid -1.5ml

PROCEDURE

Benzophenone (1.5ml), 2-amino -6-(4-dimethyl amino phenyl) -4-oxo 1,4,5,6-tetrahydropyrimidine -5-carbonitrile (1.5gm) and formic acid (1.5ml) was irradiated in microwave at 80◦C for 4minutes in equimolar quantities. The solution was obtained and then cooled in ice bath until the crystals are formed. The crude product was obtained and washed with ice cold water and air dried.

COMPOUND D - E_8



diphenylmethanone

2-amino-6-[4-(dimethylamino)phenyl]-4-oxo-1,4,5,6-tetrahydropyrimidine-5-carbonitrile

2-amino -6-[4-(dimethyl amino) phenyl]-1-(dipheny methyl) -4-oxo 1,4,5,6-tetrahydropyrimidine -5-carbonitrile

COMPOUND D –E9

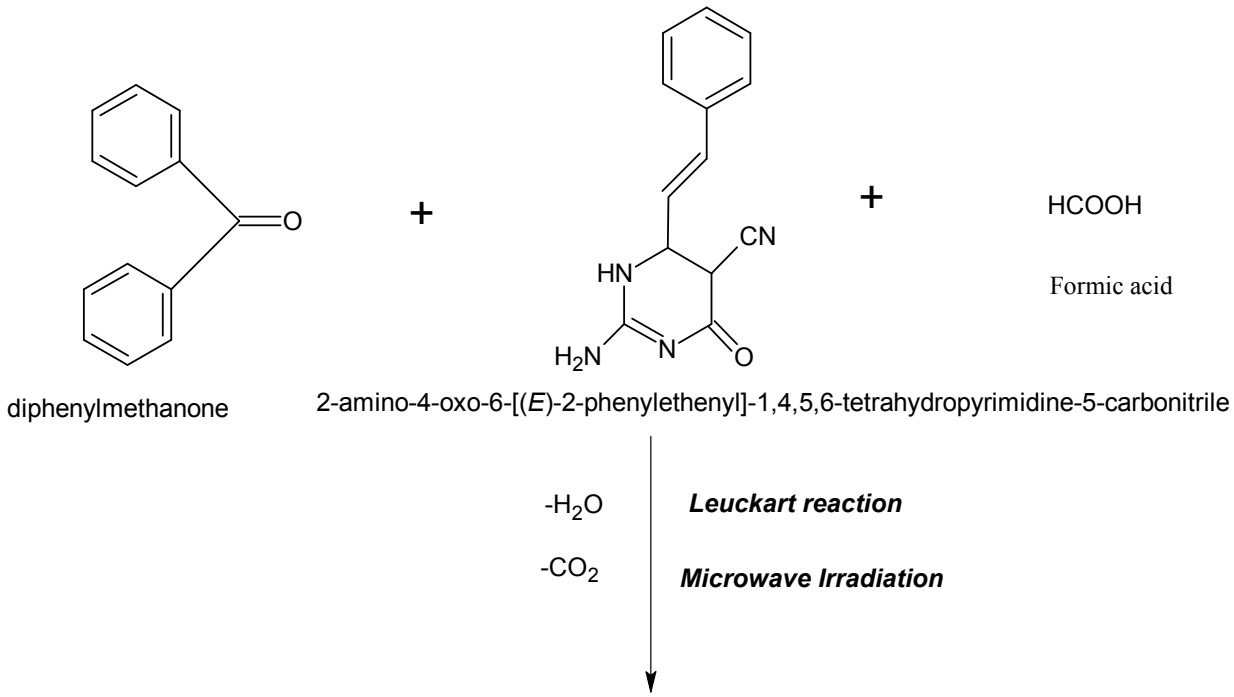
CHEMICALS REQUIRED

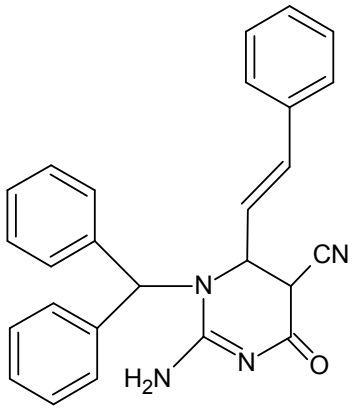
- Benzophenone -1.5ml
- 2-amino - 4- oxo -6-[(E)-2-phenyl ethenyl]
  1, 4, 5, 6-tetrahydropyrimidine -5-carbonitrile -1.5gm
- Formic acid -1.5ml

PROCEDURE

Benzophenone (1.5ml), 2-amino -4-oxo -6-[(E)-2-phenyl ethenyl] 1,4,5,6-tetrahydropyrimidine -5-carbonitrile (1.5gm) and formic acid (1.5ml) was irradiated in microwave at 80◦C for 4minutes in equimolar quantities. The solution was obtained and then cooled in ice bath until the crystals are formed. The crude product was obtained and washed with ice cold water and air dried.

COMPOUND D - E_9





2-amino -1-(diphenyl methyl) -4-oxo -6-[(E)-2-phenyl ethenyl] 1,4,5,6-tetrahydropyrimidine -5-carbonitrile

COMPOUND D –E10

CHEMICALS REQUIRED

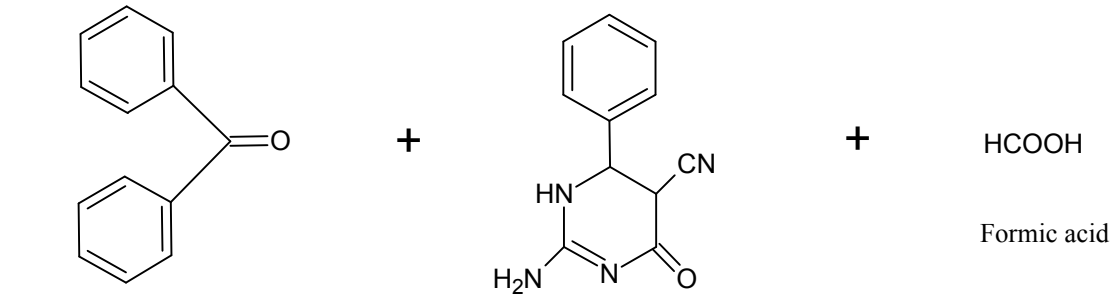
- Benzophenone -1.5ml
- 2-amino - 4- oxo -6-phenyl

  1, 4, 5, 6-tetrahydropyrimidine -5-carbonitrile -1.5gm
- Formic acid -1.5ml

PROCEDURE

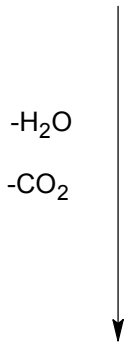
Benzophenone (1.5ml), 2-amino -4-oxo -6-phenyl 1, 4, 5, 6-tetrahydropyrimidine -5-carbonitrile (1.5gm) and formic acid (1.5ml) was irradiated in microwave at 80◦C for 4 minutes in equimolar quantities. The solution was obtained and then cooled in ice bath until the crystals are formed. The crude product was obtained and washed with ice cold water and air dried.

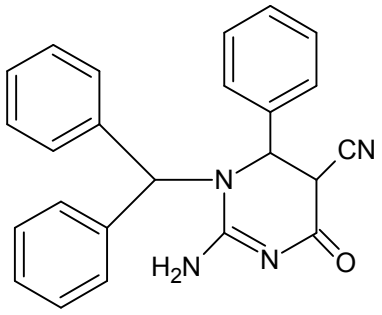
COMPOUND D - E_{10}



diphenylmethanone

2-amino-4-oxo-6-phenyl-1,4,5,6-tetrahydropyrimidine-5-carbonitrile





2-amino-1-(diphenylmethyl)-4-oxo-6-phenyl-1,4,5,6-tetrahydropyrimidine-5-carbonitrile

ANALYTICAL DATA

6. ANALYTICAL DATA OF SYNTHESIZED COMPOUNDS

PHYSICAL DATA ANALYSIS

Table No: 2

COMPOUND CODE	CHEMICAL NAME
D – E1	2-amino -6-(4-chloro phenyl)-4-oxo -1-(1-phenylethyl)1,4,5,6-tetrahydropyrimidine -5-carbonitrile
D – E2	2-amin -6-(4-methoxy phenyl) -4-oxo -1- (1-phenylethyl) 1,4,5,6-tetrahydropyrimidine -5-carbonitrile
D – E3	2-amino -6-[4-(dimethyl amino) phenyl] -4-oxo -1-(1-phenylethyl) 1,4,5,6-tetrahydropyrimidine -5-carbonitrile
D – E4	2-amino -4-oxo [(E)-2-phenylethenyl] -1-(1-phenylethyl) 1,4,5,6-tetrahydropyrimidine -5-carbonitrile
D – E5	2-amino -4-oxo -6-phenyl -1-(1-phenylethyl) 1,4,5,6-tetrahydropyrimidine -5-carbonitrile
D – E6	2-amino -6-(4-chloro phenyl) -1-(diphenylmethyl) -4-oxo 1,4,5,6-tetrahydropyrimidine -5-carbonitrile
D – E7	2-amino -1-(diphenylmethyl) -6-(4-methoxy phenyl) -4-oxo 1,4,5,6-tetrahydropyrimidine -5-carbonitrile
D – E8	2-amino -6-[4-(dimethyl amino) phenyl] -1-(diphenylmethyl) -4-oxo 1,4,5,6-tetrahydropyrimidine -5-carbonitrile
D – E9	2-amino -1-(diphenylmethyl) -4-oxo -6-[(E)-2-phenylethenyl] 1,4,5,6-tetrahydropyrimidine -5-carbonitrile
D – E10	2-amino -1-(diphenylmethyl) -4-oxo -6-phenyl 1,4,5,6-tetrahydro pyrimidine -5-carbonitrile

PHYSICAL DATA ANALYSIS

Table No: 3

COMPOUND CODE	MOLECULAR FORMULA	MOLECULAR WEIGHT
D – E1	C19H17ClN4O	352.82
D – E2	C20H20N4O2	348.39
D – E3	C21H23N5O	361.44
D – E4	C21H20N4O	344.40
D – E5	C19H18N4O	318.37
D – E6	C24H19ClN4O	414.88
D – E7	C25H22N4O2	410.46
D – E8	C26H27N5O	425.52
D – E9	C26H22N4O	406.47
D – E10	C24H20N4O	380.44

PHYSICAL DATA ANALYSIS

Table No: 4

COMPOUD CODE	SOLUBILIY	APPEARANCE/ COLOUR	PERCENGE YIELD
D – E1	$CHCl_3$	SOLID/PALE YELLOW	75%
D – E2	$CHCl_3$	SOLID/YELLOW	68%
D – E3	$CHCl_3$	SOLID/YELLOW	82%
D – E4	$CHCl_3$	SOLID/ORANGE	62%
D – E5	$CHCl_3$	SOLID/YELLOW	55%
D – E6	$CHCl_3$	SOLID/YELLOW	80%
D – E7	$CHCl_3$	SOLID/YELLOW	64%
D – E8	$CHCl_3$	SOLID/YELLOW	74%
D – E9	$CHCl_3$	SOLID/PALE YELLOW	58%
D – E10	$CHCl_3$	SOLID/YELLOW	67%

ELEMENTAL ANALYSIS

Table No: 5

COMPOUND CODE	%C	%H	%N	%O	% Cl
D – E1	64.68%	4.86%	15.88%	4.53%	10.05
D – E2	68.95%	15.79%	16.08%	9.18%	-
D – E3	69.78%	6.41%	19.38%	4.43%	-
D – E4	73.23%	5.85%	16.27%	4.65%	-
D – E5	71.68%	5.7%	17.6%	5.03	-
D – E6	69.48%	4.62%	13.3%	3.86	8.55%
D – E7	73.15%	5.4%	13.65%	7.8%	-
D – E8	73.39%	6.4%	16.46%	3.76%	-
D – E9	76.83%	5.46%	13.78%	3.94%	-
D – E10	75.77%	5.3%	14.73%	4.21%	-

MELTING POINT ANALYSIS

Melting point was determined by using open end capillary tube.

The melting point of synthesized compounds is given in the **Table No: 6**

S.NO	COMPOUND	MELTING POINT ^{0}C
1	D – E1	174
2	D – E 2	180
3	D – E3	236
4	D – E4	240
5	D – E5	225
6	D – E6	252
7	D – E7	263
8	D – E8	245
9	D – E9	208
10	D – E10	196

THIN LAYER CHROMATOGRAPHY

The principle of separation is adsorption. Thin layer chromatography was carried out by using silica gel(0.5mm thickness) coated over the glass plate (12x20 cm) as stationary phase, $CHCl_3$: CH_3OH (9:1) or $CHCl_3$: C_2H_5OH (6:4) as mobile phase and spots were visualized by iodine vapours.

Mobile Phase used

Chloroform : Methanol

9 : 1

Table No: 7

COMPOUND CODE	R_f VALUE
D – E1	0.74
D – E2	0.68
D – E3	0.75
D – E4	0.65
D – E5	0.64
D – E6	0.67
D – E7	0.70
D – E8	0.72
D – E9	0.63
D – E10	0.71

SPECTRAL ANALYSIS

7. SPECTRAL ANALYSIS[14, 49]

7.1 IR SPECTROSCOPY

- The range of electromagnetic radiation between 0.8µ and 500µ is termed as infrared radiation. The IR spectrum is represented with percent transmittance (%T) in the ordinate and the wave number (cm^{-1}) in the abscissa.
- The IR radiation refers to broadly to that region of electromagnetic spectrum which lies between visible and microwave region. IR region is divided into 3 sections
  - i) Mid IR region – wave length 25µ to 2.5µ
  - ii) Near IR region – wave length 0.8µ to 2.5µ
  - iii) Far IR region - wave length 25µ to1000µ
- The most commonly used region of IR spectrum in pharmaceutical chemistry between 4000- 400 cm^{-1}
- IR spectroscopy is one of the most powerful analytical techniques which offer the possibility of chemical identification. One of the most advantages of IR over the other usual methods of structural analysis (X-ray diffraction, electron spin resonance etc.) is that it provides useful information about the structure molecule quickly.
- IR spectroscopy can solve many problems in organic chemistry and coordination chemistry.
- IR spectroscopy can be used identification of functional group, drug substance and impurities in a drug sample.

PELLET TECHNIQUE

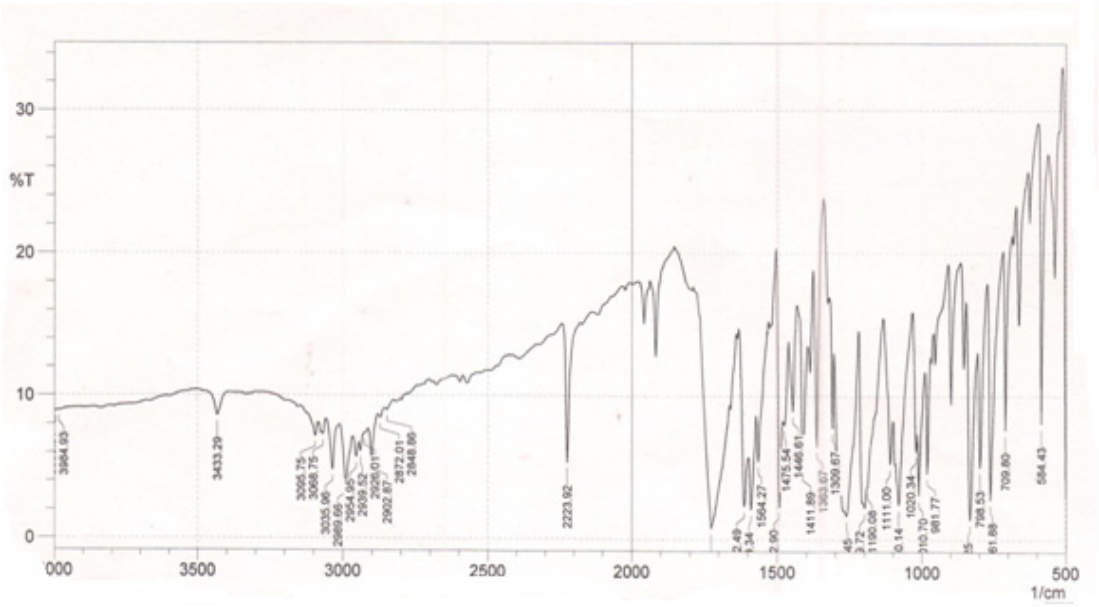
The solid sample is mixed with powdered potassium bromide and triturate in a smooth mortar. The mixture is uniformly spread over the dye and compressed into a thin transparent pellet using hydraulic press under pressure 15000 psi. The IR spectral data of the synthesized compounds were recorded on Fourier Tranform IR spectrometer in the ranges of 4000 - 400 cm^{-1} and the values are reported.

INFRARED DATA (TableNo: 8)

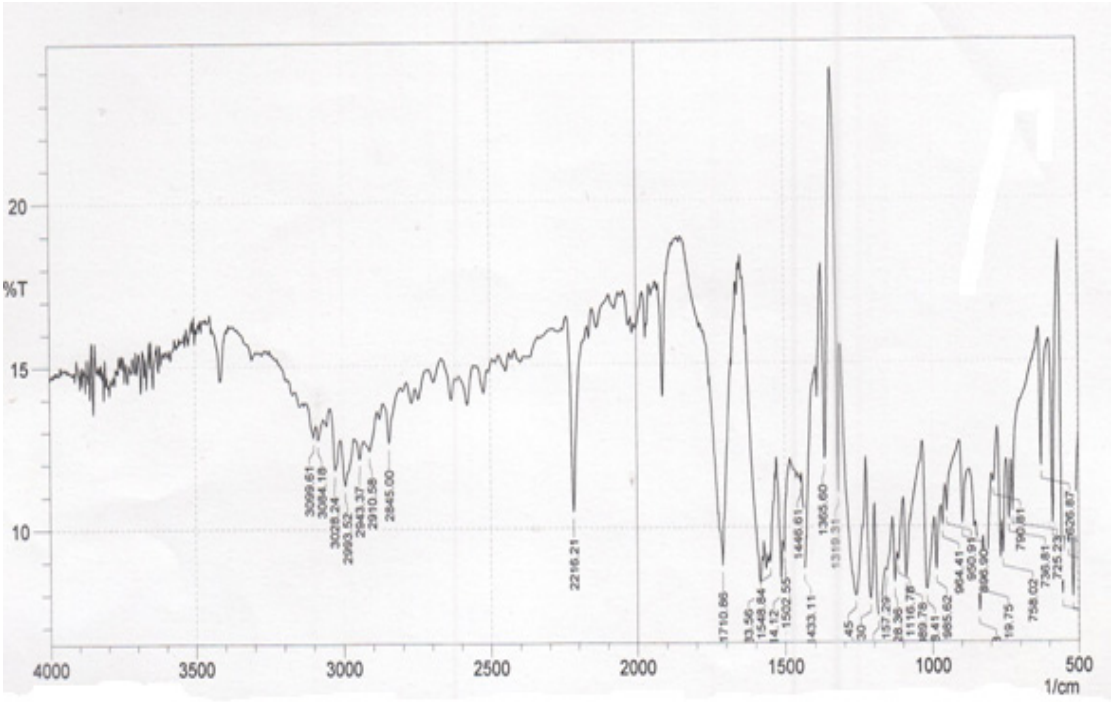
COMPOUND CODE	FUNCTIONAL REGION	FREQUENCY(cm^{-1})
D – E1	C= O str (Ketone)	1612
	N-H str($Ar1^0$ Amine)	3433
	C=N str	1446
	C≡N str (Nitrile)	2223
	C- N str	1309
	C-H str (methyl)	2872
	Ar-Cl	709
D – E2	C= O str (Ketone)	1637
	N-H str($Ar1^0$ Amine)	3368
	C=N str	1502
	C≡N str (Nitrile)	2216
	C-N str	1275
	C-H str (methyl)	2943
	OCH_3	2834
D – E3	C= O str (Ketone)	1660
	N-H str($Ar1^0$ Amine)	3730
	C=N str	1431
	C≡N str (Nitrile)	2208
	C-N str	1371
	C-H str (methyl)	2860
	$N(CH_3)_2$	1327
D – E4	C= O str (Ketone)	1612
	N-H str($Ar1^0$ Amine)	3300
	C=N str	1448
	C≡N str (Nitrile)	2220
	C-Nstr	1085
	C-H str (methyl)	2852
	C=Cstr (alkene)	1658
	=CH str	3134
D – E5	C= O str (Ketone)	1598
	N-H str($Ar1^0$ Amine)	3311
	C=N str	1444
	C≡N str (Nitrile)	2223
	C-Nstr	1010
	C-H str (methyl)	2850
	Ar-H str	

D –E6	C= O str(Ketone)	1658
	N-H str(Ar1^0 Amine)	3315
	C=N str	1481
	C≡N str (Nitrile)	2223
	C-N str	1001
	Ar-Cl	638
D – E7	C= O str(Ketone)	1654
	N-H str(Ar1^0 Amine)	3414
	C=N str	1598
	C≡N str (Nitrile)	2216
	C-N str	1018
	OCH_3	846
D – E8	C= O str(Ketone)	1548
	N-H str(Ar1^0 Amine)	3458
	C=N str	1562
	C≡N str (Nitrile)	2250
	C-N str	1064
	$N(CH_3)_2$	1317
D – E9	C= O str(Ketone)	1610
	N-H str(Ar1^0 Amine)	3380
	C=N str	1597
	C≡N str (Nitrile)	2220
	C-N str	1085
	C=C str (alkene)	1658
	=CH str	3028
D – E10	C= O str(Ketone)	1658
	N-H str(Ar1^0 Amine)	3309
	C=N str	1442
	C≡N str (Nitrile)	2223
	C-N str	1010
	Ar-H str	3001

D – E1


%T
30
20
10
0
000
3500
3000
2500
2000
1500
1000
500
1/cm
3964.93
3433.29
3095.75
3068.75
3035.96
2980.66
2964.95
2939.52
2925.01
2902.87
2872.01
2848.86
2223.92
2.49
1.34
1564.27
2.90
1475.54
1446.61
1411.89
1383.67
1309.67
45
3.72
1190.08
1111.00
0.14
1020.34
910.70
981.77
15
798.53
61.88
709.80
584.43

D – E2


%T
20
15
10
4000
3500
3000
2500
2000
1500
1000
500
1/cm
3099.61
3084.18
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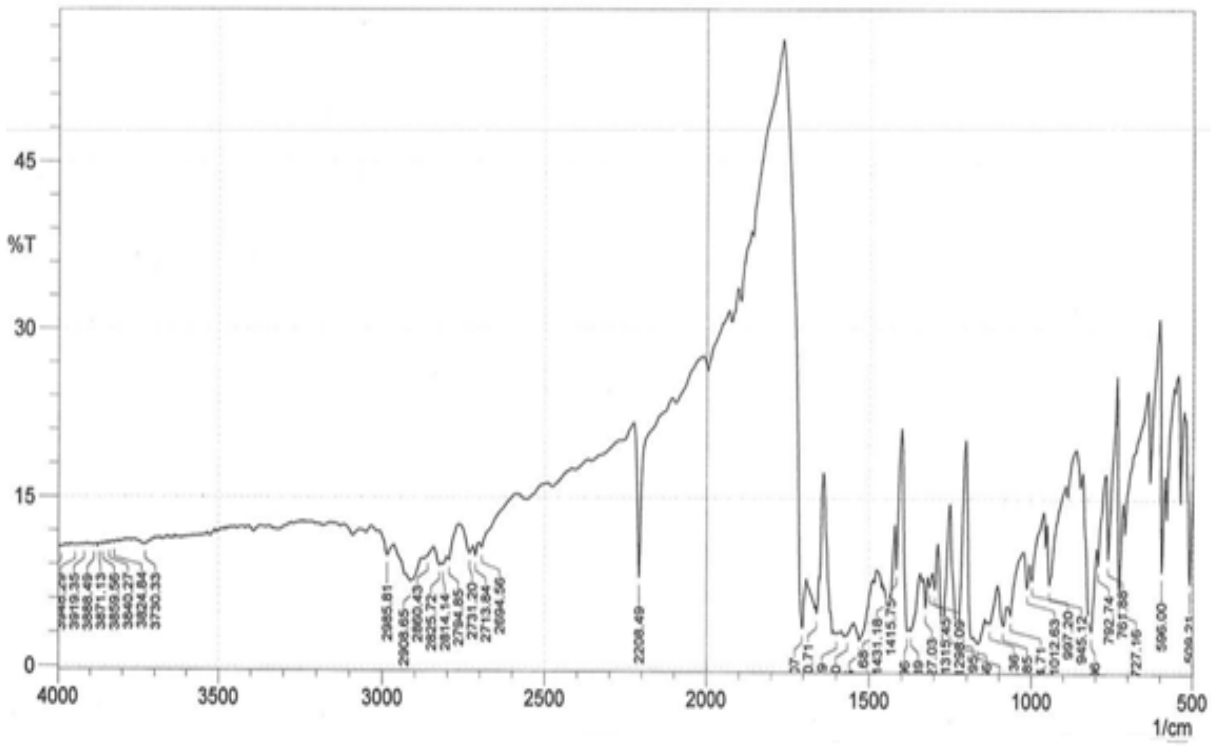
2993.52
2943.37
2910.58
2845.00
2216.21
1710.86
33.56
1548.84
14.12
1502.55
1446.61
1433.11
1365.60
1316.31
45
30
17.29
16.36
1116.78
980.78
9.41
985.62
964.41
950.91
936.90
19.75
790.81
758.02
736.81
725.23
626.87

D – E3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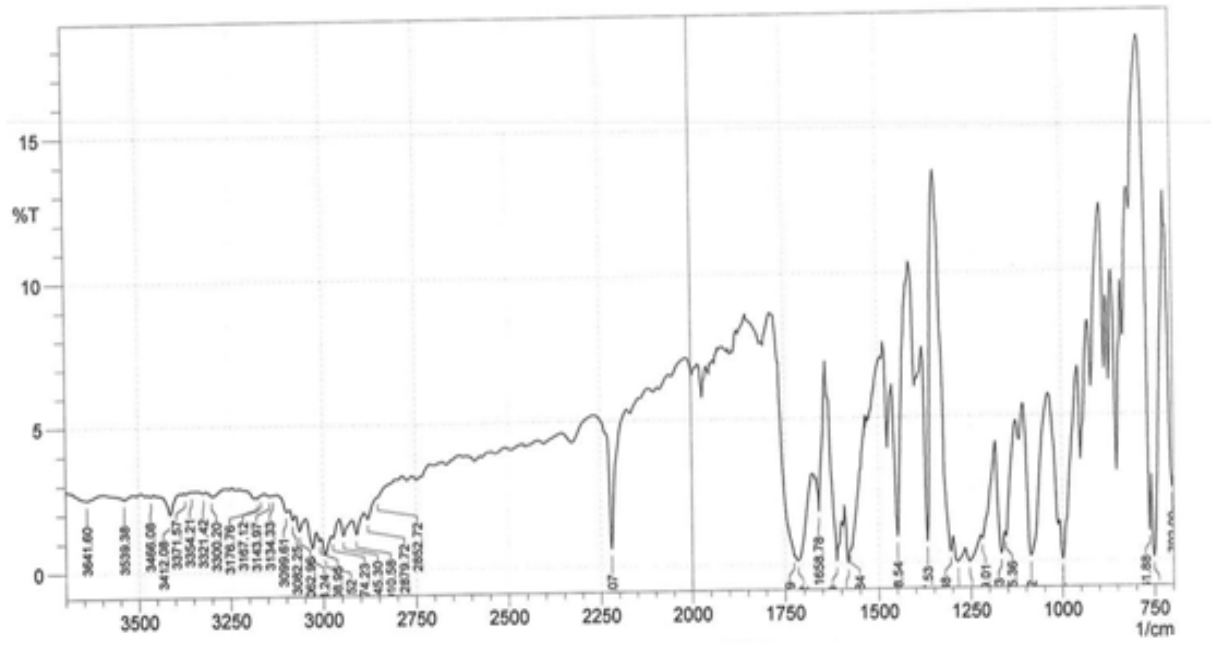
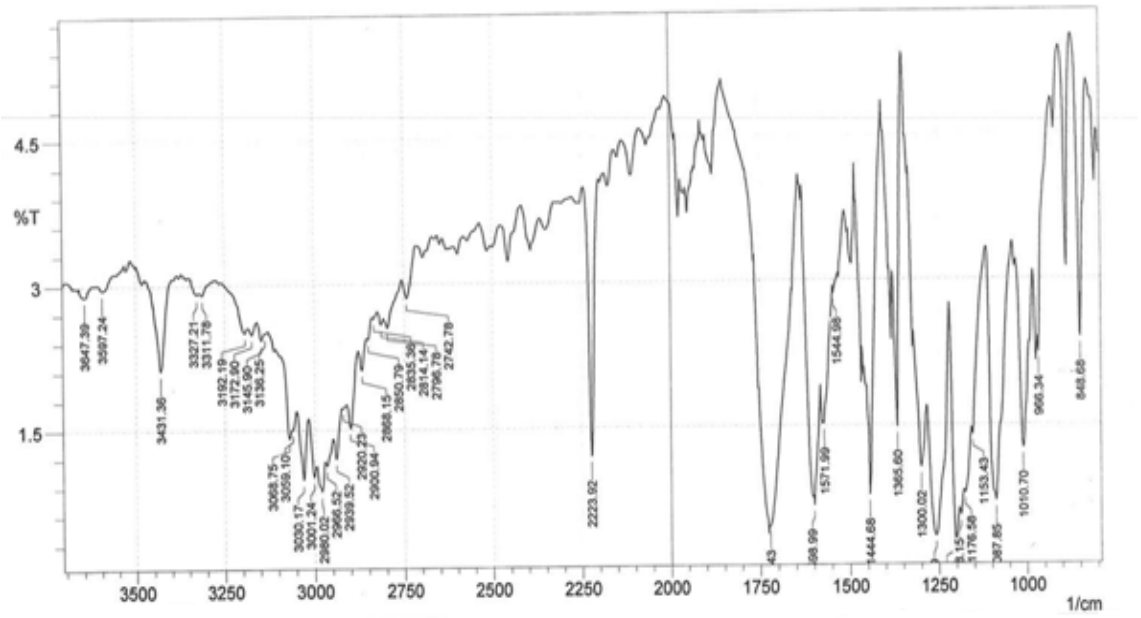
%T
30
15
0
4000
3500
3000
2500
2000
1500
1000
500
1/cm

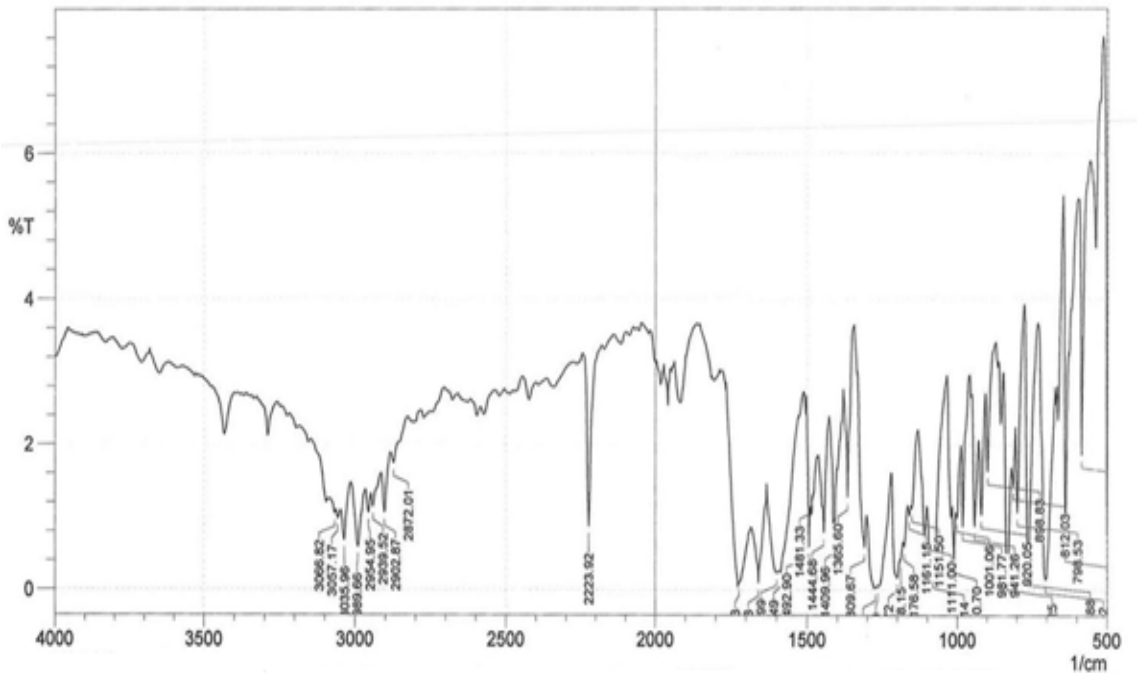
D – E4


15
%T
10
5
0
3500
3250
3000
2750
2500
2250
2000
1750
1500
1250
1000
750
1/cm

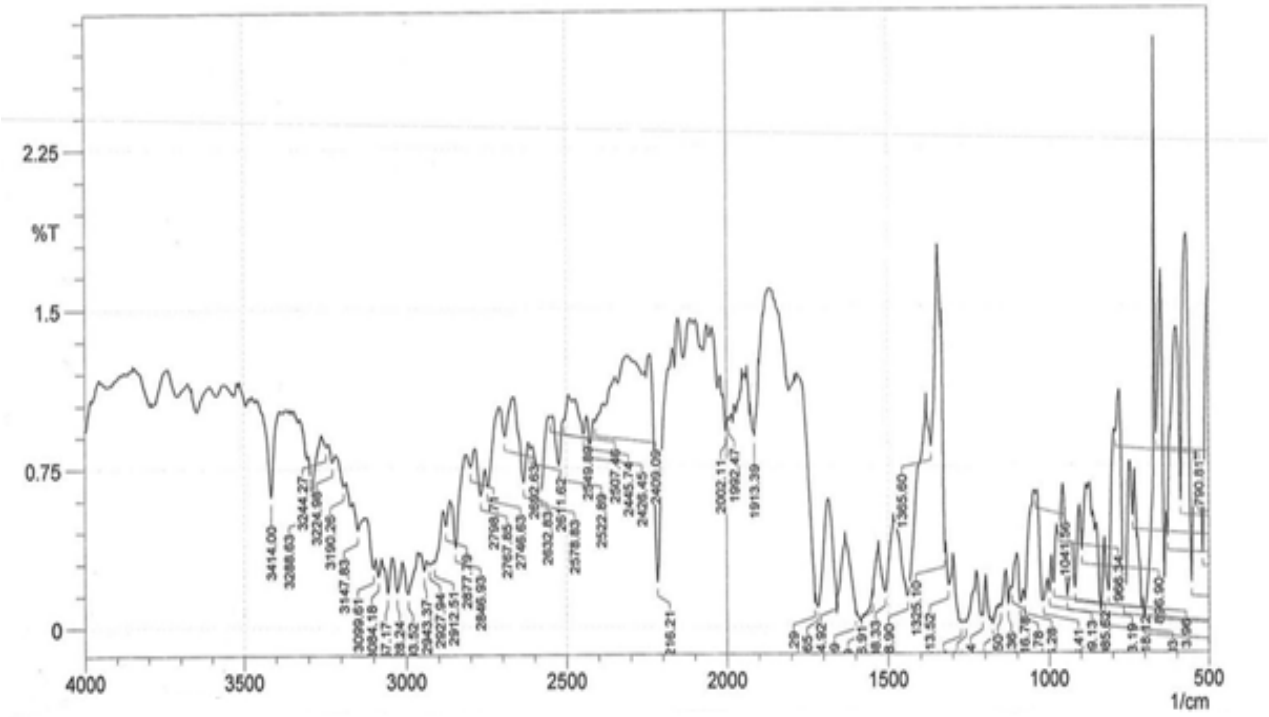
D – E5


4.5
%T
3
1.5
3500
3250
3000
2750
2500
2250
2000
1750
1500
1250
1000
1/cm

D – E6


6
%T
4
2
0
4000
3500
3000
2500
2000
1500
1000
500
1/cm

D – E7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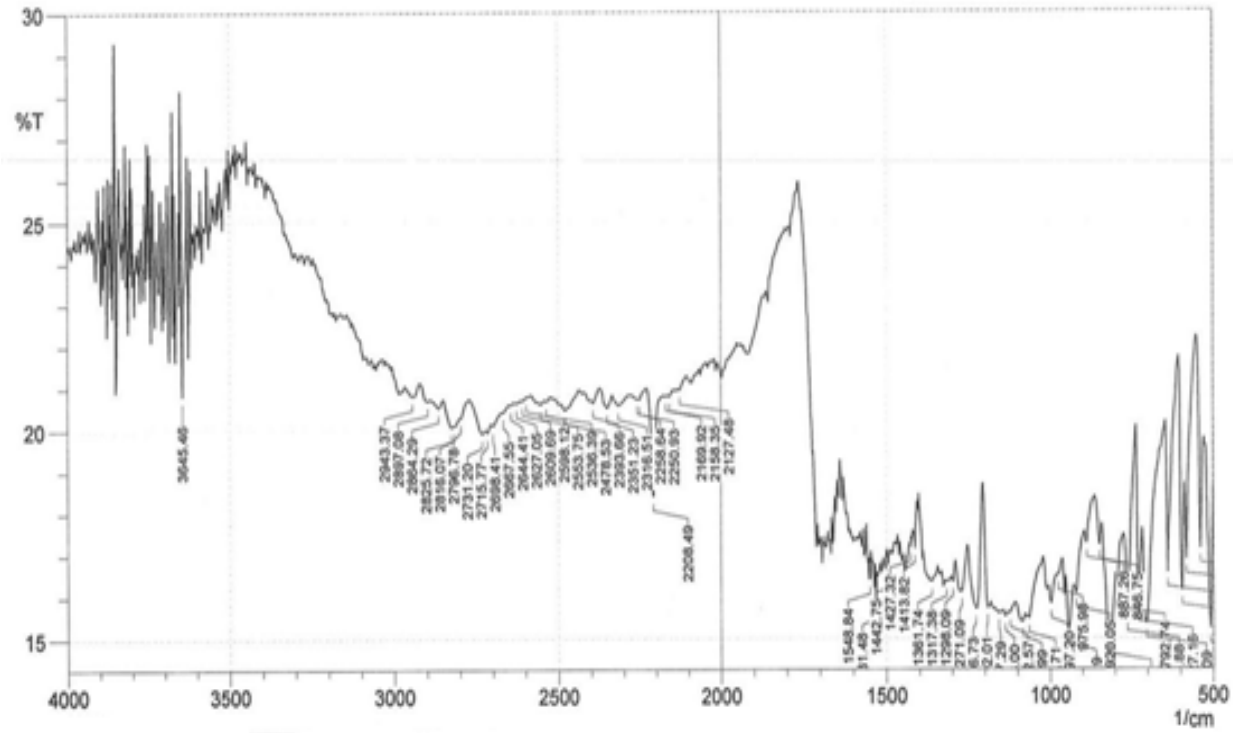
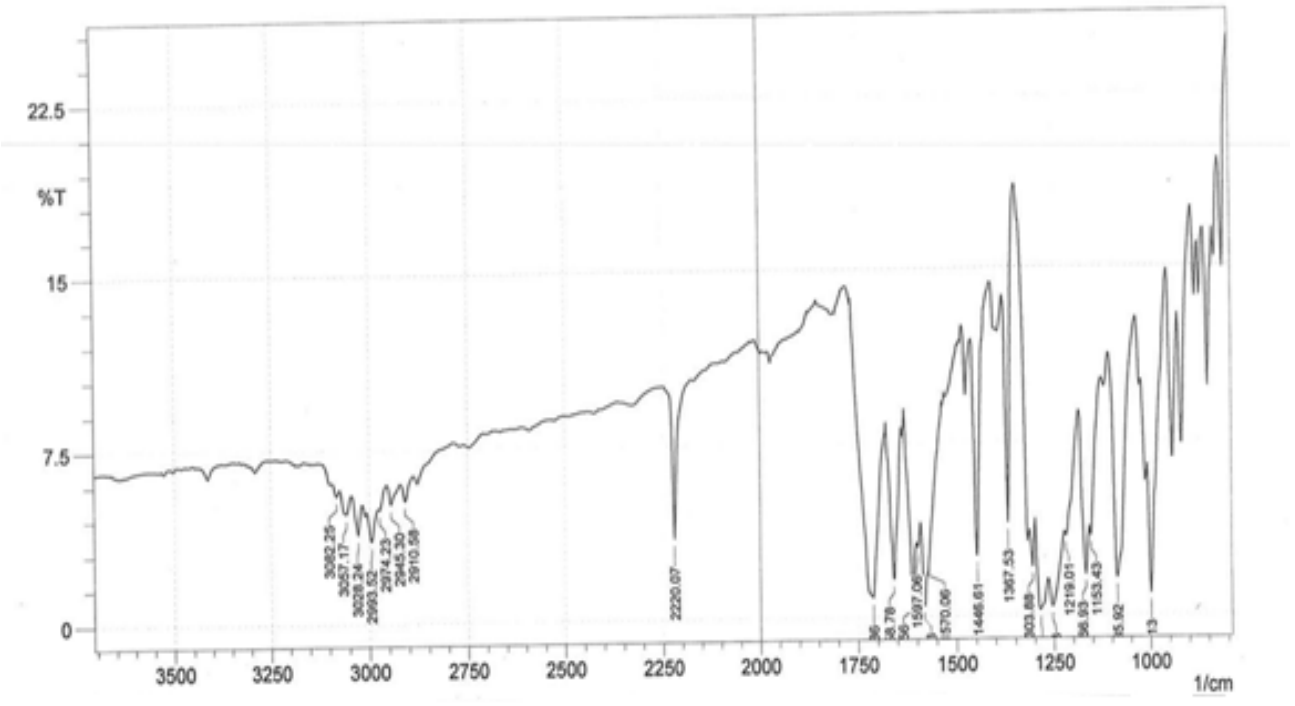
%T
1.5
0.75
0
4000
3500
3000
2500
2000
1500
1000
500
1/cm

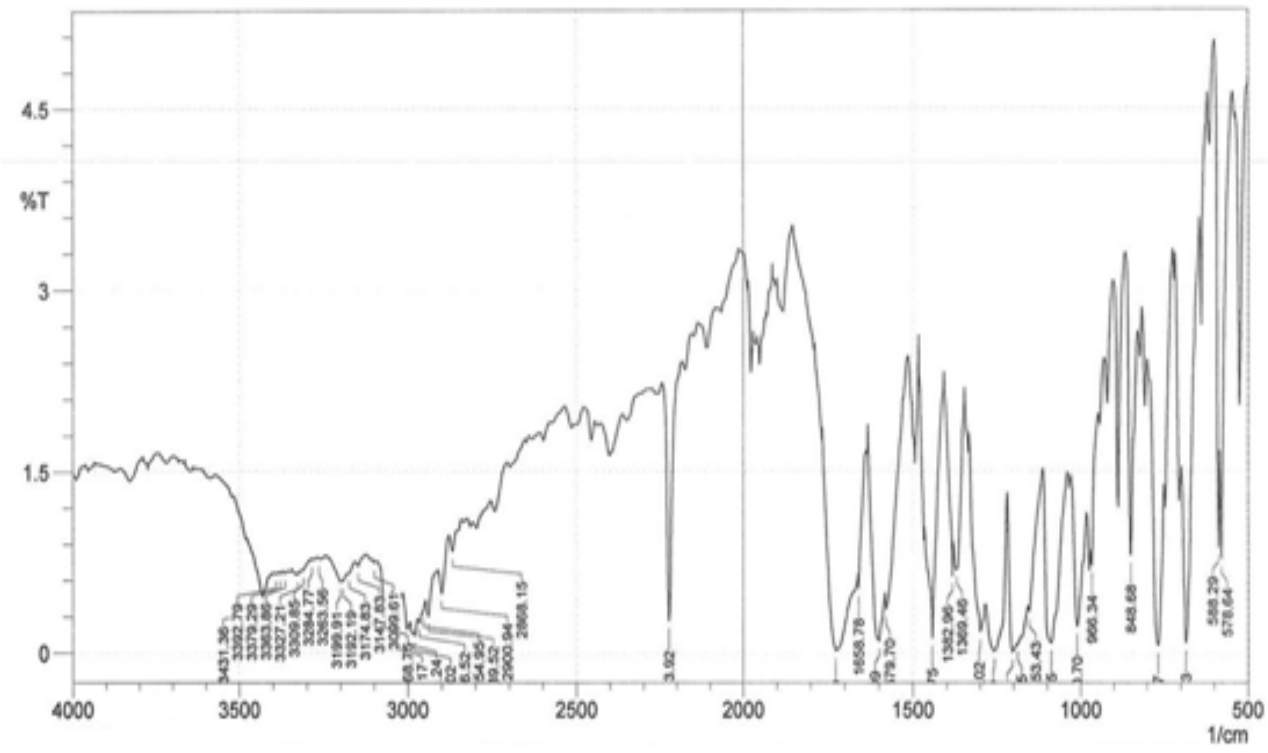
D – E8


30
%T
25
20
15
4000
3500
3000
2500
2000
1500
1000
500
1/cm

D- E9


22.5
%T
15
7.5
0
3500
3250
3000
2750
2500
2250
2000
1750
1500
1250
1000
1/cm
3082.25
3057.17
3028.24
2993.52
2974.23
2945.30
2910.58
2220.07
1597.06
1570.06
1446.61
1367.53
1219.01
1153.43

D – E10


4.5
%T
3
1.5
0
4000
3500
3000
2500
2000
1500
1000
500
1/cm
3431.36
3392.79
3379.29
3363.86
3327.21
3309.85
3284.77
3261.56
3199.91
3182.19
3114.83
3147.83
3099.61
2868.15
2900.94
1658.78
1382.96
1369.46
966.34
848.68
588.29
578.64

MASS SPECTROSCOPY

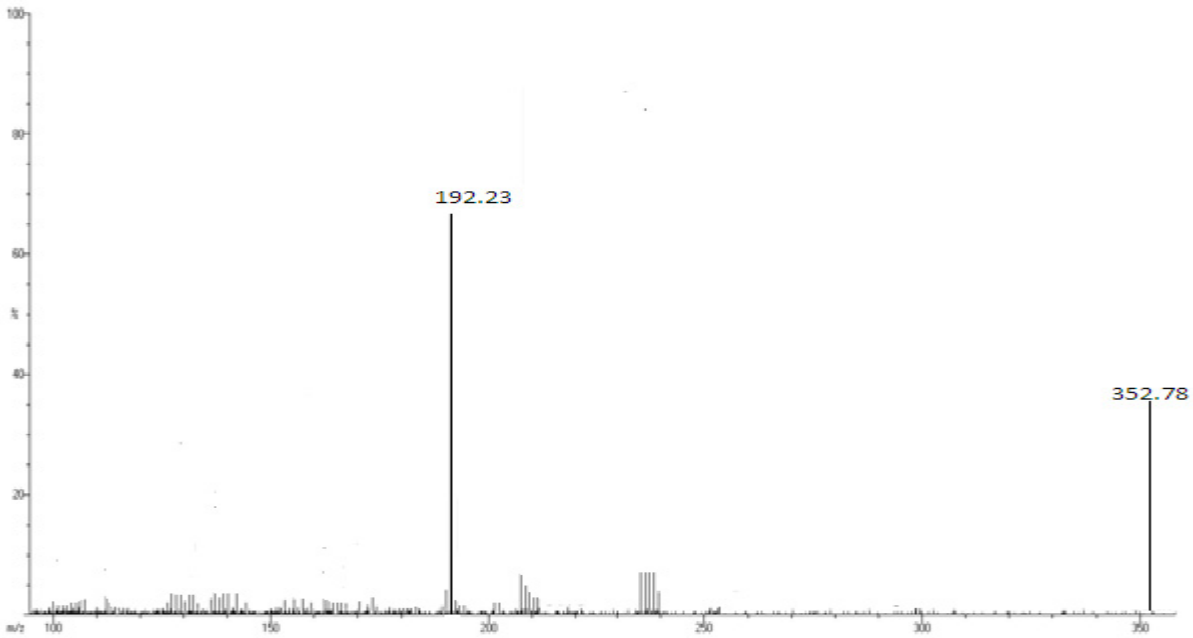
- Mass spectroscopy is an analytical techniques which can provide more information Concerning the molecular mass of organic and inorganic compounds.
- Mass spectroscopy is most accurate, speed, reliability and expensive instrument.
- In this technique, molecules are bambarded with a beam of energetic electrons which produce an ionic molecule. The resulting assortment of charged particles is then separated according to their masses and some ions which are positive ions.
- Each kind of ions has particular ratio of mass to charge, i.e-m/e ratio. The molecular ion is called as parent ion and the largest peak in the structure is called as base peak.
- The m/e value of the parent ion is equal to the molecular mass of the compound. Molecular ion peaks are recorded in m/e ratio.

MASS SPECTRAL DATA

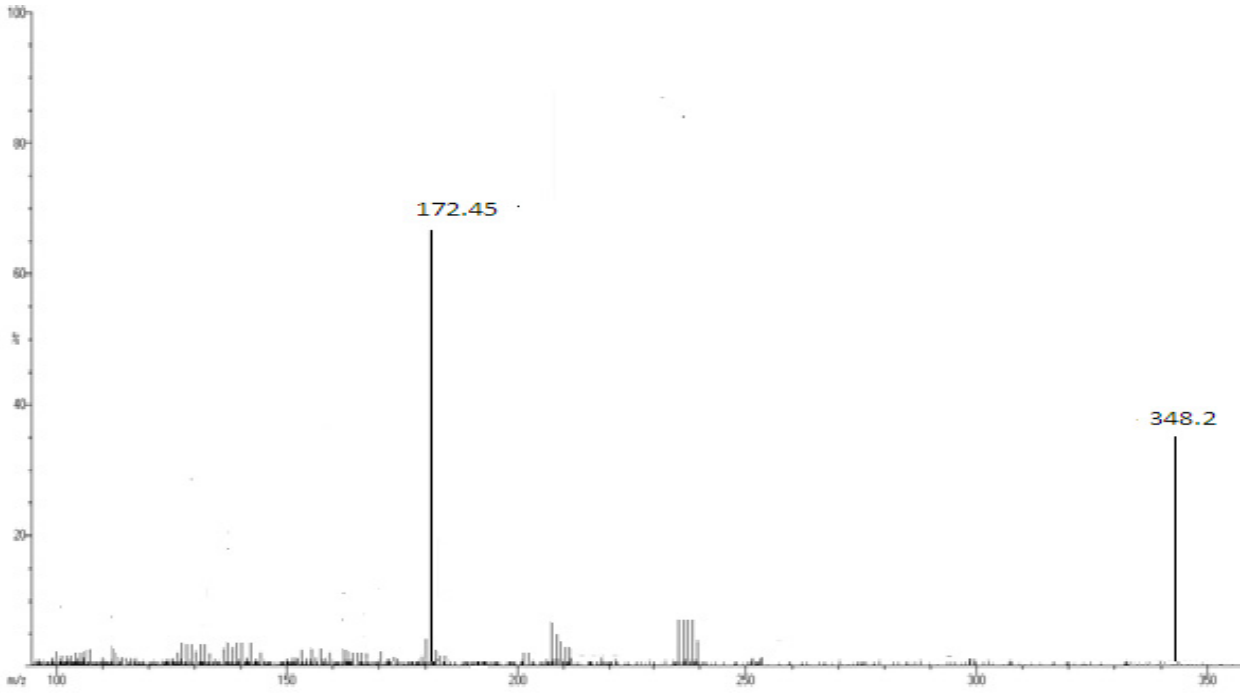
Table No: 9

COMPOUND CODE	MOLECULAR IONS
D – E1	352.82
D – E2	348.39
D – E3	361.44
D – E4	344.40
D – E5	318.37
D – E6	414.88
D – E7	410.46
D – E8	425.52
D – E9	406.47
D – E10	380.44

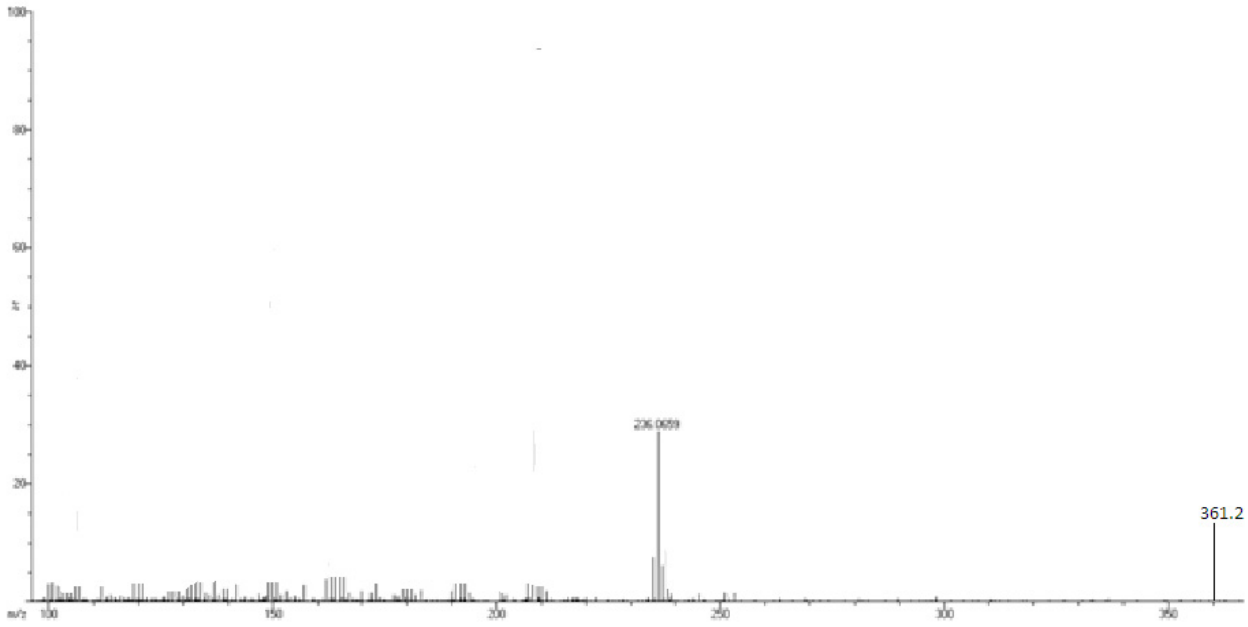
D – E1



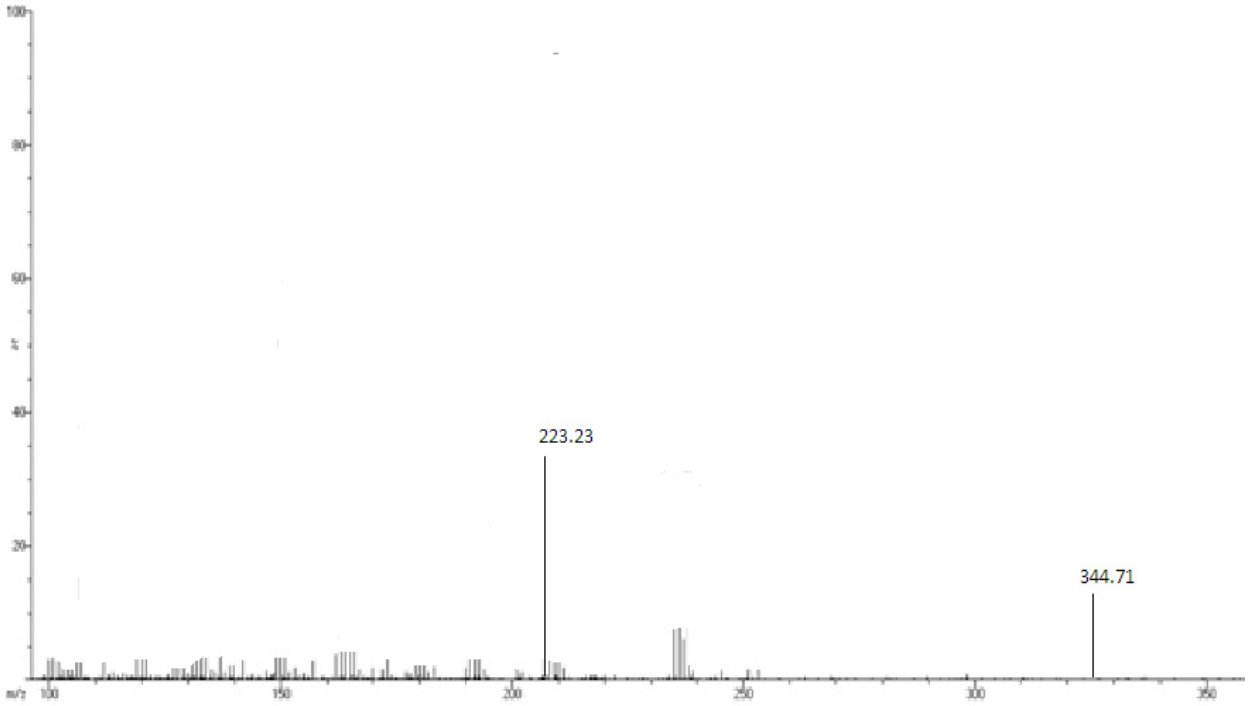
D – E2



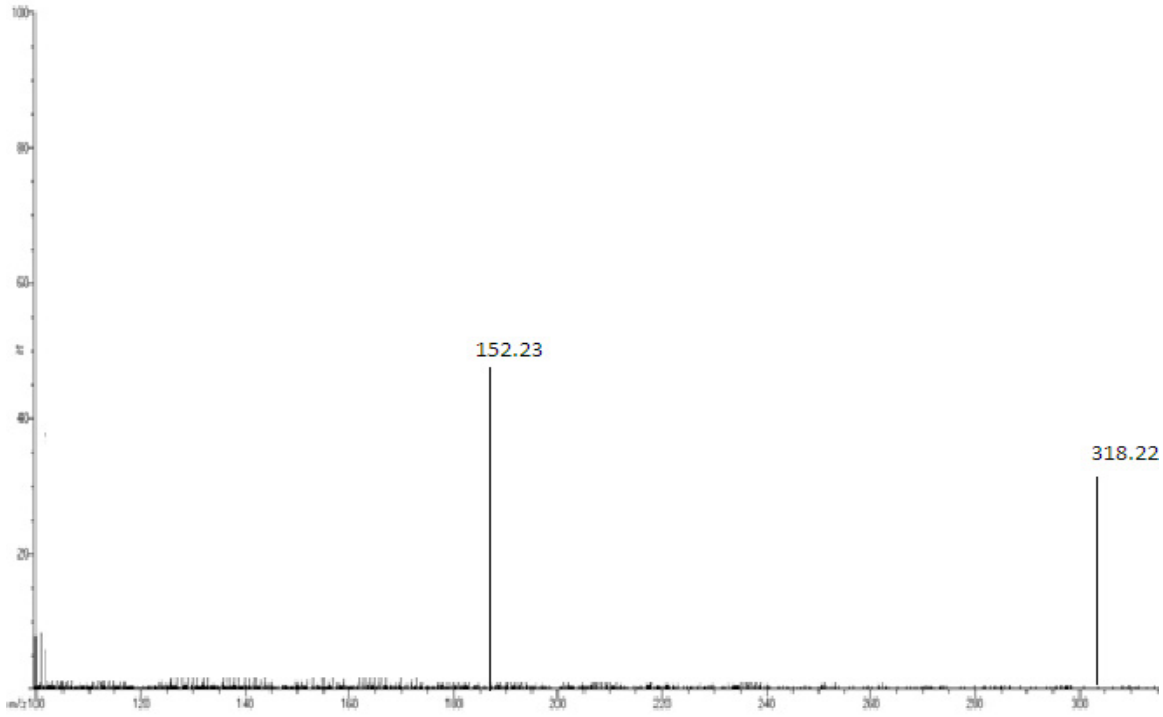
D – E3



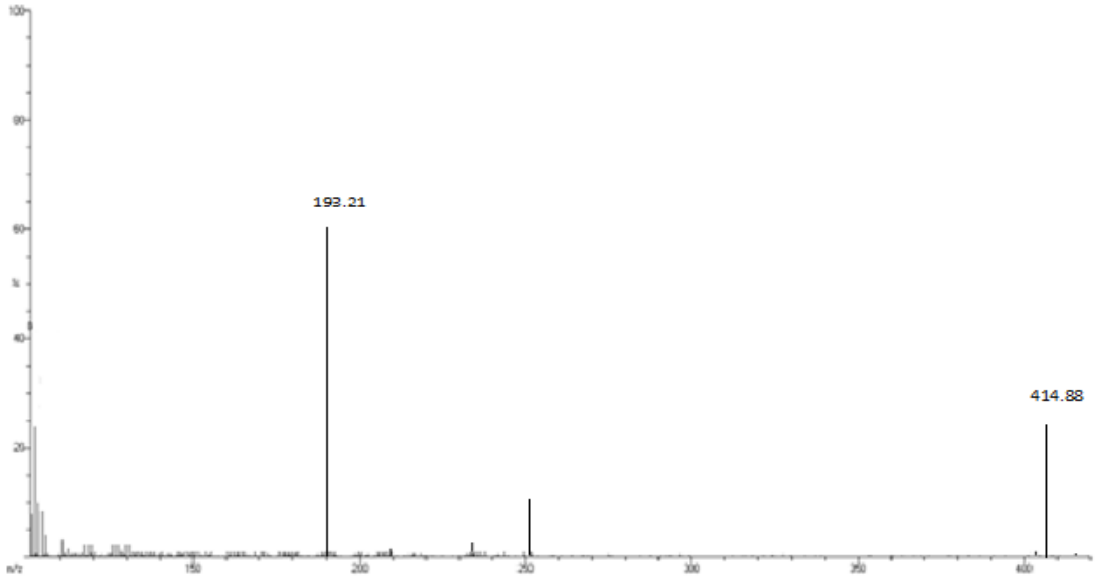
D – E4



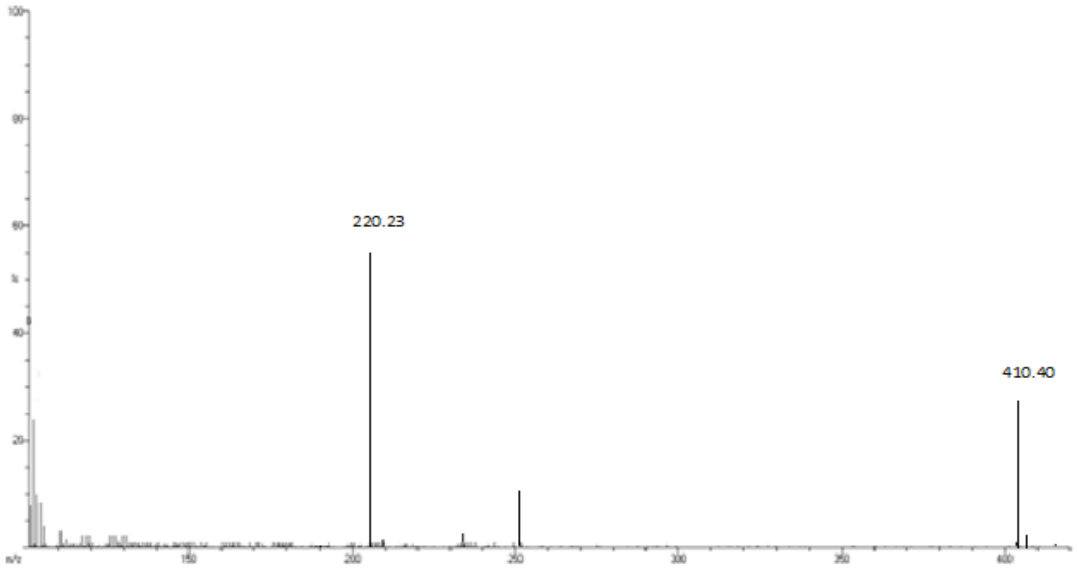
D – E5



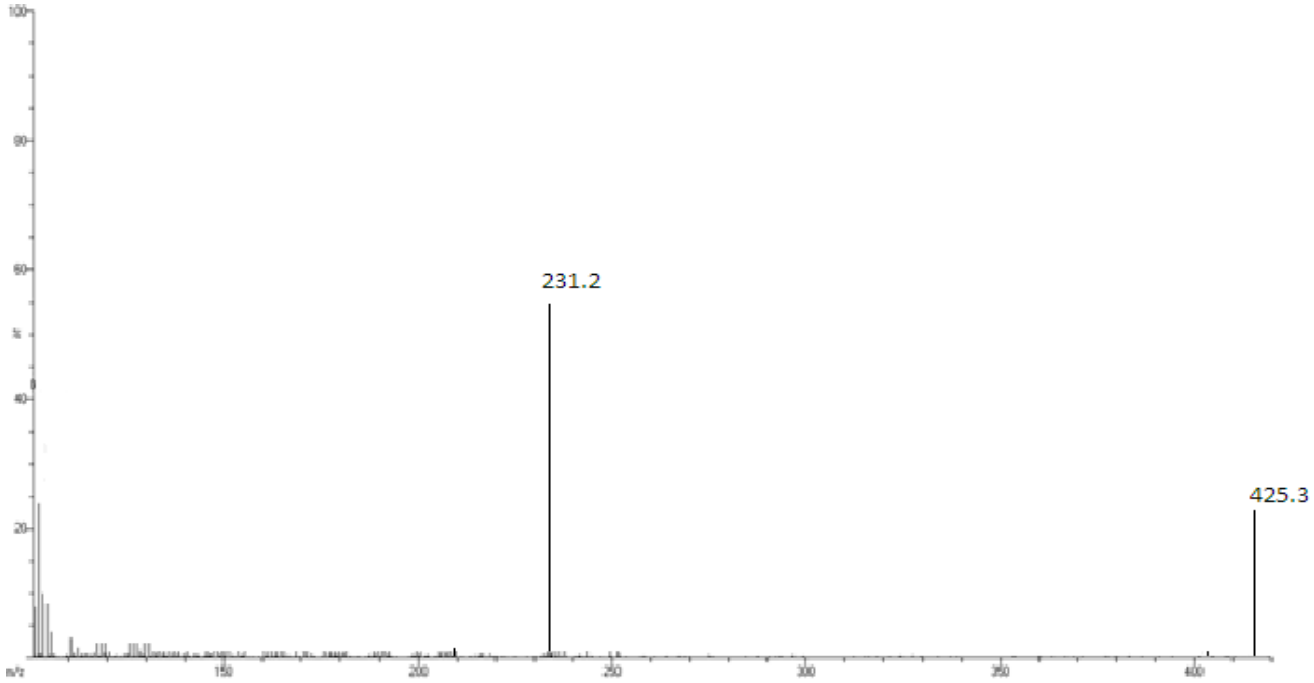
D – E6



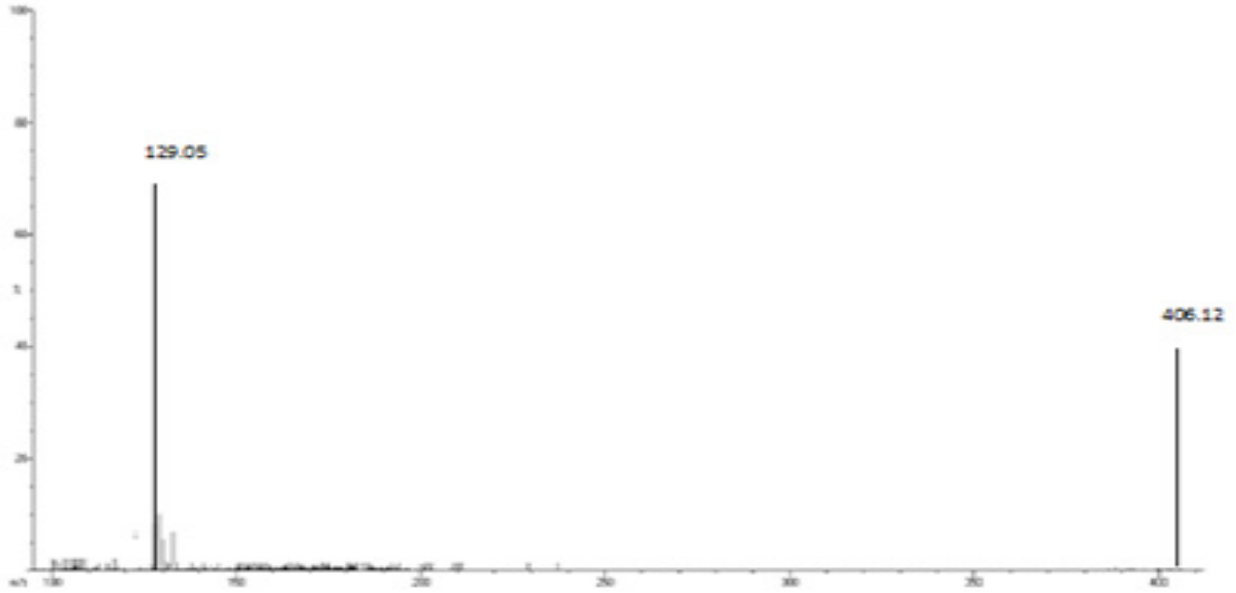
D – E7



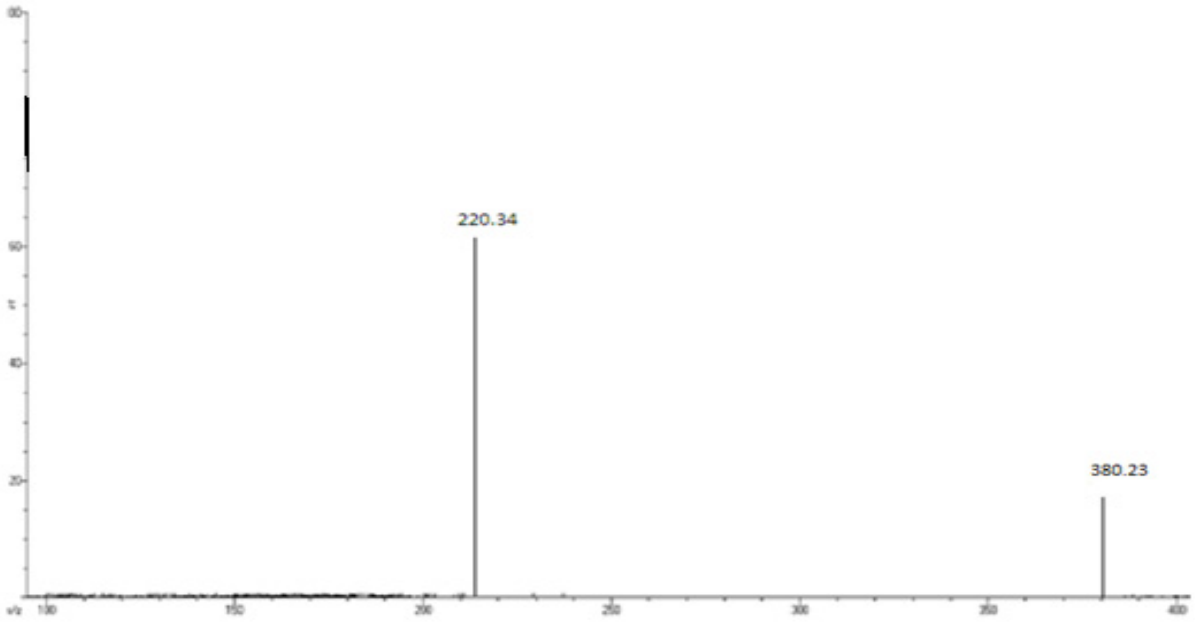
D – E8



D – E9



D – E10



7.3. NMR SPECTROSCOPY

- NMR spectroscopy is an analytical method for determining the structural elucidation of organic compounds and quantitative analysis included in hydrogen analysis, moisture analysis, iodine value.
- Nuclear Magnetic Resonance spectroscopy is the study of spin changes at the nuclear level when radio frequency energy is absorbed in the presence of magnetic field. When proton (hydrogen) is studied then it is called as proton magnetic resonance.
- Proton or Nuclei with odd mass number only gives NMR spectra. eg: ^{1}H, ^{13}C, ^{19}F, ^{35}Cl etc.
- The solvent used in the NMR spectroscopy should not contain hydrogen atoms. Hence we use solvents like carbon tetrachloride, Deuterated chloroform, Deuterated water, Deuterated dimethysulphoxide, Deuterated acetic acid, Deuterated triflouro acetic acid.
- Any proton or nucleus with odd mass number spinning on its own axis by the application of an external magnetic field and radio frequency energy. When absorption of energy occurs and a NMR signal is recorded.
- A combination of 60MHz radio frequency and a magnetic field strength of 14,092gauss is applied for high resolution instruments, other combination is used.
- ^{1}H NMR spectra were recorded on Bruker – NMR 400MHz using DMSO and chemical shifts were reported in parts per million and Tetra Methyl Silane is used as reference standard in NMR spectroscopy.
- Chemical shift is the difference between the absorption position of the sample proton and absorption position of the reference compound. Chemical shift is measured in δ value and normal ranges from o - 10δ.

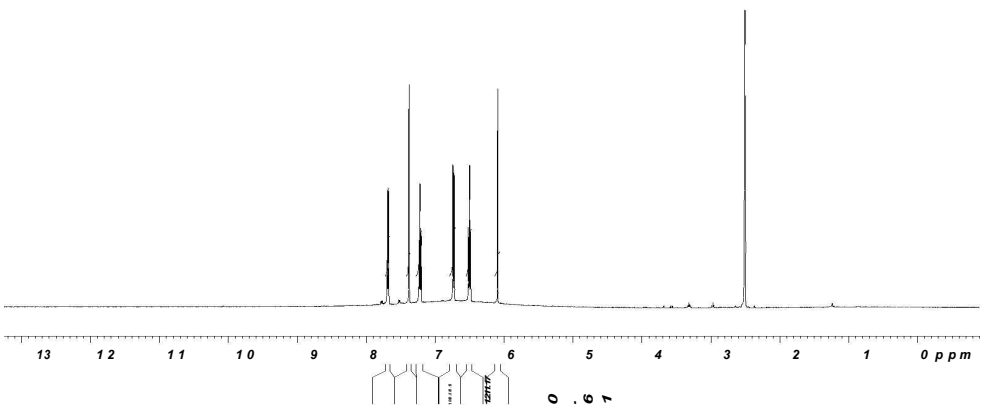
NMR SPECTRAL DATA

Table No: 10

COMPOUND CODE	PROTON NATURE	CHEMICAL SHIFT
D – E1	NH_2 (2H) CH_3 (3H) CH-CN (1H) CH-N (1H) Ar- H (9H) CH-Cl (1H)	2.5 1.2 2.9 2.2 7.4 3.3
D – E2	NH_2 (2H) CH_3 (3H) CH-CN (1H) CH-N (1H) Ar- H (9H) OCH_3 (3H)	3.4 2.5 2.5 3.5 7.3 3.7
D – E3	NH_2(2H) CH_3 (3H) CH-CN (1H) CH-N (1H) Ar- H (9H) $N(CH3)_2$	2.2 1.3 2.5 4.2 7.6 6.5
D – E4	NH_2(2H) CH_3 (3H) CH-CN (1H) CH-N (1H) Ar- H (10H) CH =CH	2.7 1.2 2.4 3.9 7.4 2.1
D – E 5	NH_2(2H) CH_3 (3H) CH-CN (1H) CH-N (1H) Ar- H (10H)	2.5 1.3 2.7 2.5 7.8

D – E6	NH_2 (2H) CH-CN (1H) CH-N (1H) Ar- H (14H) CH-Cl (1H	2.6 2.8 2.1 7.3 3.2
D – E7	NH_2 (2H) CH-CN (1H) CH-N (1H) Ar- H (14H) OCH_3 (3H)	3.3 2.6 2.2 7.2 3.7
D – E8	NH_2(2H) CH-CN (1H) CH-N (1H) Ar- H (14H) $N(CH3)_2$ (6H)	2.1 2.6 4.3 7.5 6.5
D – E9	NH_2(2H) CH-CN (1H) CH-N (1H) Ar- H (15H) CH =CH (2H)	2.5 2.8 3.8 7.2 2.1
D – E10	NH_2(2H) CH-CN (1H) CH-N (1H) Ar- H (15H)	3.1 2.6 2.2 7.7

D – E1



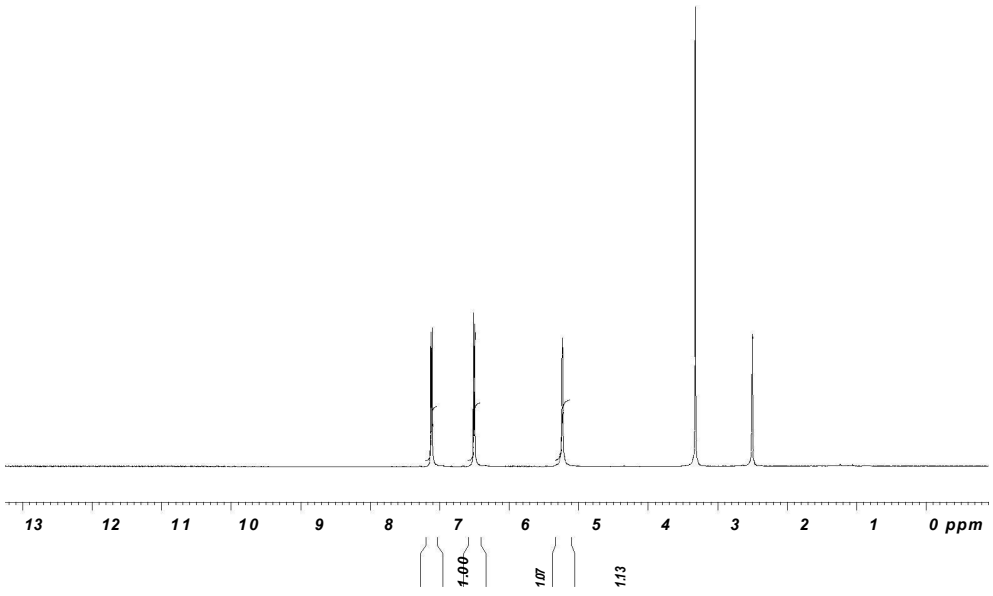

```
Current Data Parameters
NAME          Feb11-2014
EXPNO                5
PROCNO               1

F2 - Acquisition Parameters
Date_         20140211
Time              21.49
INSTRUM           spect
PROBHD    5 mm PABBO BB-
PULPROG           zg30
TD               32768
SOLVENT           DMSO
NS                  32
DS                   2
SWH         10330.578 Hz
FIDRES       0.315264 Hz
AQ          1.5860212 sec
RG                 203
DW           48.400 usec
DE            6.50 usec
TE            300.0 K
D1          1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
NUC1                 1H
P1             10.65 usec
PL1             0.00 dB
PL1W       23.53637505 W
SFO1       500.1330885 MHz

F2 - Processing parameters
SI               32768
SF         500.1300000 MHz
WDW                 EM
SSB                  0
LB              0.30 Hz
GB                   0
PC              1.00
```

D – E2



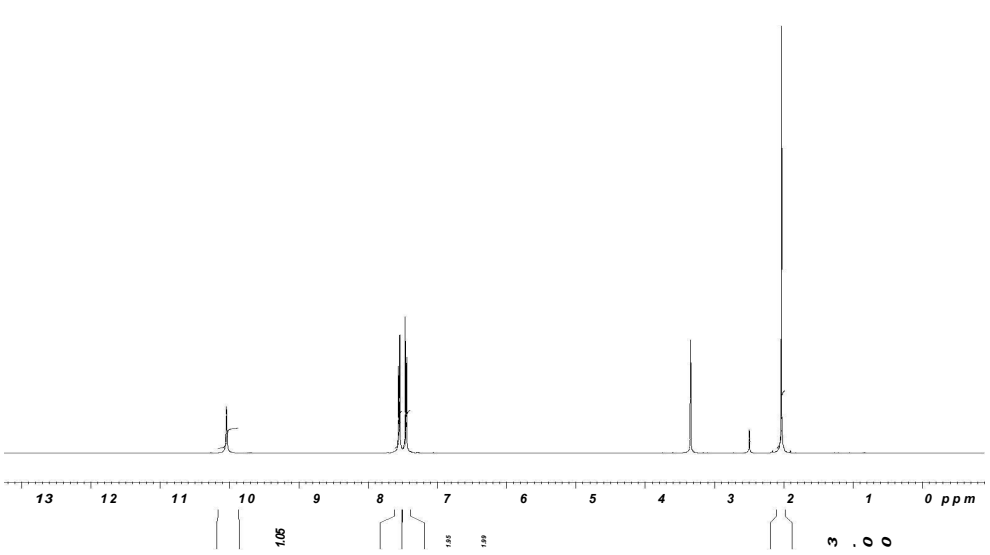

```
Current Data Parameters
NAME          Feb11-2014
EXPNO                26
PROCNO               1

F2 - Acquisition Parameters
Date_         20140211
Time              10.47
INSTRUM           spect
PROBHD    5 mm PABBO BB-
PULPROG           zg30
TD               32768
SOLVENT           DMSO
NS                  32
DS                   2
SWH         10330.578 Hz
FIDRES       0.315264 Hz
AQ          1.5860212 sec
RG                 203
DW           48.400 usec
DE            6.50 usec
TE            299.2 K
D1          1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
NUC1                 1H
P1             10.65 usec
PL1             0.00 dB
PL1W       23.53637505 W
SFO1       500.1330885 MHz

F2 - Processing parameters
SI               32768
SF         500.1300036 MHz
WDW                 EM
SSB                  0
LB              0.30 Hz
GB                   0
PC              1.00
```

D – E3



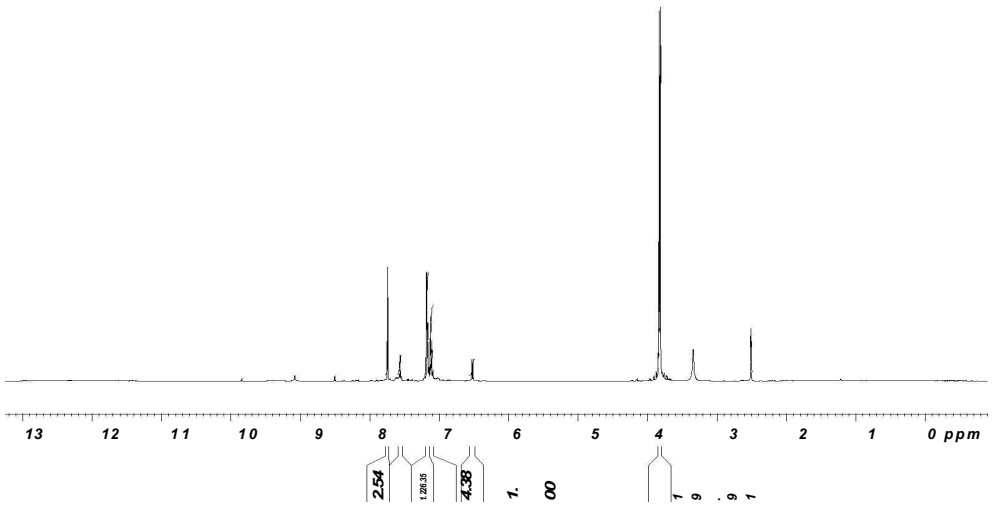

```
Current Data Parameters
NAME          Feb11-2014
EXPNO                7
PROCNO               1

F2 - Acquisition Parameters
Date_         20140211
Time          21.56
INSTRUM         spect
PROBHD   5 mm PABBO BB-
PULPROG          zg30
TD              32768
SOLVENT          DMSO
NS                  32
DS                   2
SWH         10330.578 Hz
FIDRES       0.315264 Hz
AQ          1.5860212 sec
RG                 161
DW           48.400 usec
DE            6.50 usec
TE            300.1 K
D1          1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
NUC1                 1H
P1             10.65 usec
PL1             0.00 dB
PL1W       23.53637505 W
SFO1        500.1330885 MHz

F2 - Processing parameters
SI              32768
SF         500.1300039 MHz
WDW                EM
SSB                 0
LB             0.30 Hz
GB                  0
PC             1.00
```

D – E4



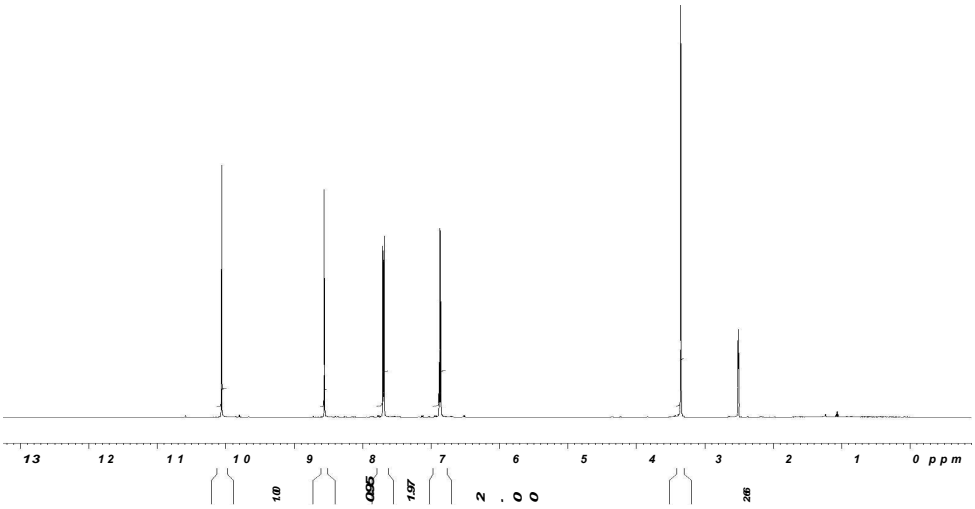

```
Current Data Parameters
NAME          Feb11-2014
EXPNO                2
PROCNO               1

F2 - Acquisition Parameters
Date_         20140211
Time          21.39
INSTRUM         spect
PROBHD   5 mm PABBO BB-
PULPROG          zg30
TD              32768
SOLVENT          DMSO
NS                  32
DS                   2
SWH         10330.578 Hz
FIDRES       0.315264 Hz
AQ          1.5860212 sec
RG                 181
DW           48.400 usec
DE            6.50 usec
TE            299.9 K
D1          1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
NUC1                 1H
P1             10.65 usec
PL1             0.00 dB
PL1W       23.53637505 W
SFO1        500.1330885 MHz

F2 - Processing parameters
SI              32768
SF         500.1300000 MHz
WDW                EM
SSB                 0
LB             0.30 Hz
GB                  0
PC             1.00
```

D – E5



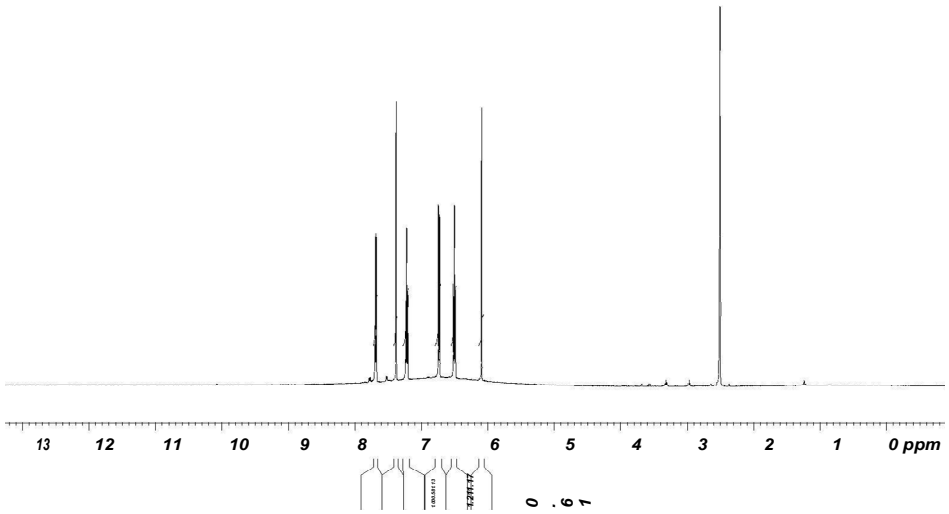
Current Data Parameters
NAME Feb11-2014
EXPNO 12
PROCNO 1

F2 - Acquisition Parameters
Date_ 20140211
Time 22.13
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 32768
SOLVENT DMSO
NS 32
DS 2
SWH 10330.578 Hz
FIDRES 0.315264 Hz
AQ 1.5860212 sec
RG 203
DW 48.400 usec
DE 6.50 usec
TE 300.1 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.65 usec
PL1 0.00 dB
PL1W 23.53637505 W
SFO1 500.1330885 MHz

F2 - Processing parameters
SI 32768
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

D –E6



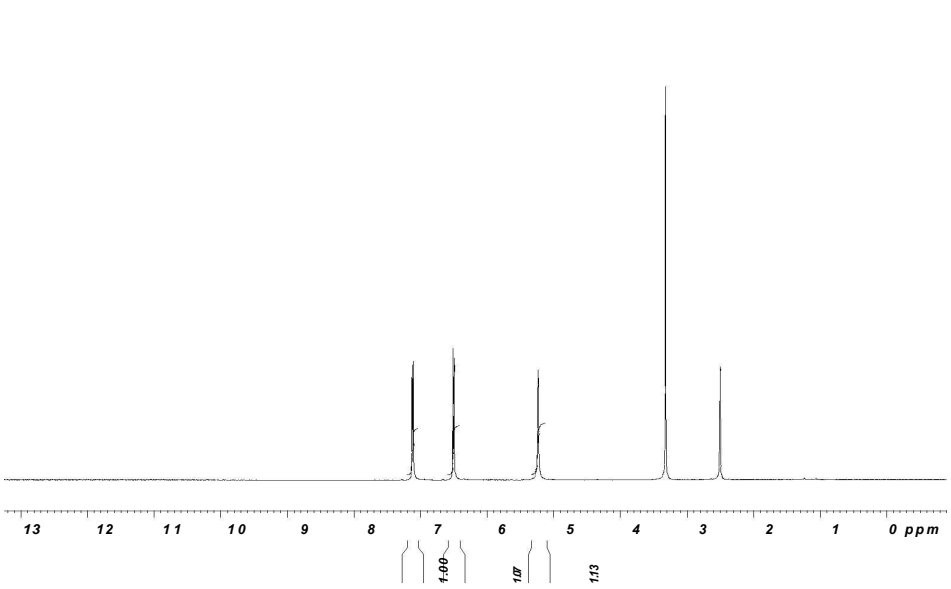
Current Data Parameters
NAME Feb11−2014
EXPNO 5
PROCNO 1

F2 − Acquisition Parameters
Date_ 20140211
Time 21.49
INSTRUM spect
PROBHD 5 mm PABBO BB−
PULPROG zg30
TD 32768
SOLVENT DMSO
NS 32
DS 2
SWH 10330.578 Hz
FIDRES 0.315264 Hz
AQ 1.5860212 sec
RG 203
DW 48.400 usec
DE 6.50 usec
TE 300.0 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.65 usec
PL1 0.00 dB
PL1W 23.53637505 W
SFO1 500.1330885 MHz

F2 − Processing parameters
SI 32768
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

D – E7



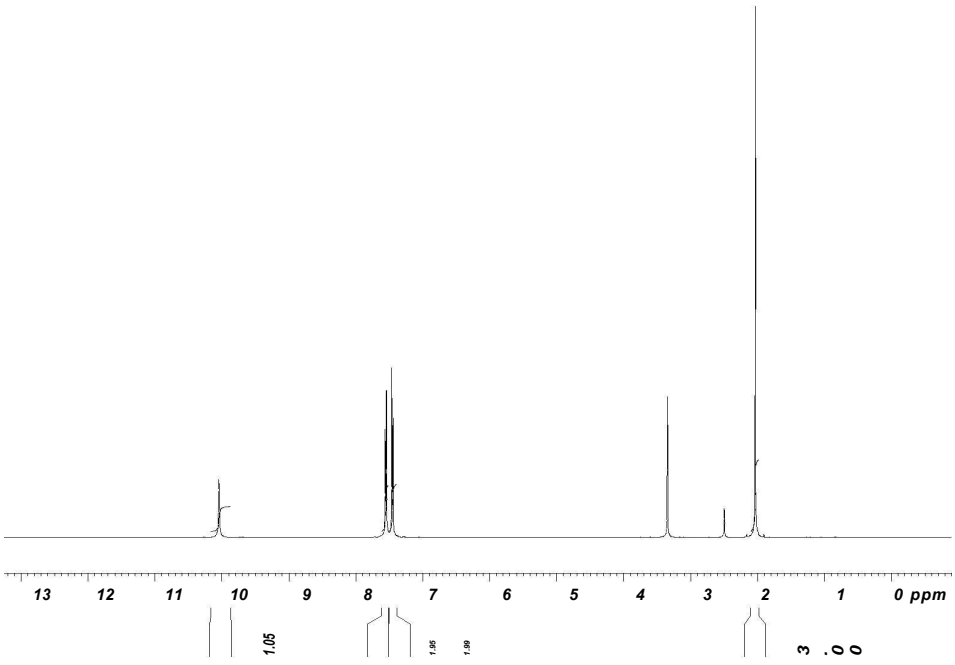
Current Data Parameters
NAME Feb11-2014
EXPNO 26
PROCNO 1

F2 - Acquisition Parameters
Date_ 20140211
Time 10.47
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 32768
SOLVENT DMSO
NS 32
DS 2
SWH 10330.578 Hz
FIDRES 0.315264 Hz
AQ 1.5860212 sec
RG 203
DW 48.400 usec
DE 6.50 usec
TE 299.2 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.65 usec
PL1 0.00 dB
PL1W 23.53637505 W
SFO1 500.1330885 MHz

F2 - Processing parameters
SI 32768
SF 500.1300036 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

D – E8



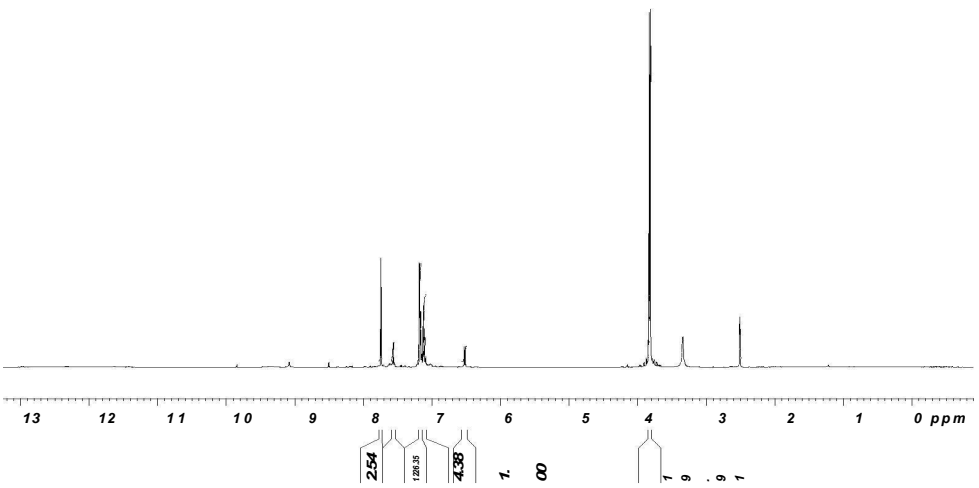
Current Data Parameters
NAME Feb11-2014
EXPNO 7
PROCNO 1

F2 - Acquisition Parameters
Date_ 20140211
Time 21.56
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 32768
SOLVENT DMSO
NS 32
DS 2
SWH 10330.578 Hz
FIDRES 0.315264 Hz
AQ 1.5860212 sec
RG 161
DW 48.400 usec
DE 6.50 usec
TE 300.1 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.65 usec
PL1 0.00 dB
PL1W 23.53637505 W
SFO1 500.1330885 MHz

F2 - Processing parameters
SI 32768
SF 500.1300039 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

D – E9



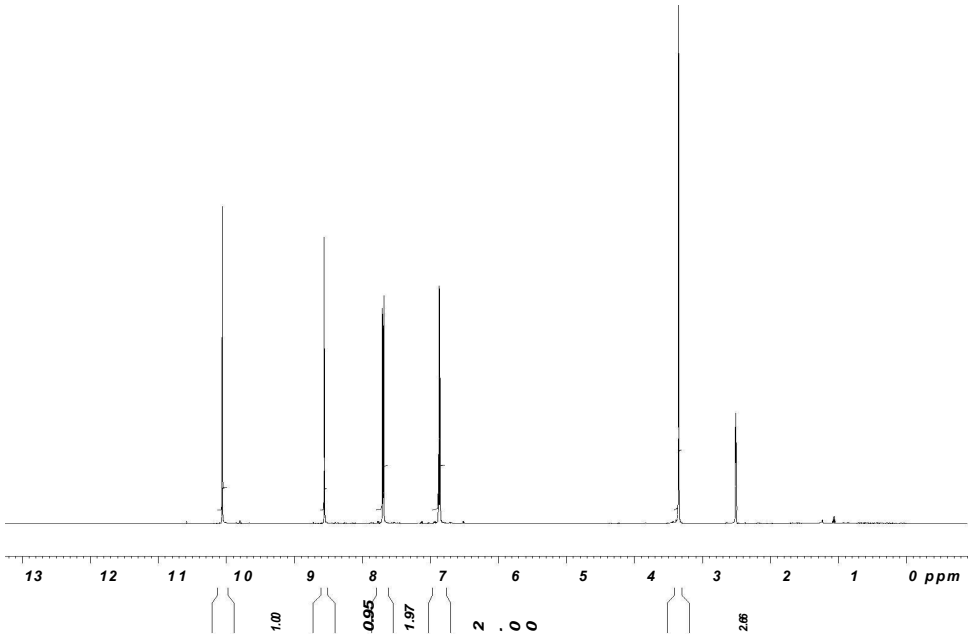

```
Current Data Parameters
NAME          Feb11-2014
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_         20140211
Time            21.39
INSTRUM          spect
PROBHD   5 mm PABBO BB-
PULPROG          zg30
TD              32768
SOLVENT          DMSO
NS                 32
DS                  2
SWH         10330.578 Hz
FIDRES       0.315264 Hz
AQ          1.5860212 sec
RG                181
DW             48.400 usec
DE              6.50 usec
TE              299.9 K
D1          1.00000000 sec
TD0                1

======== CHANNEL f1 ========
NUC1               1H
P1             10.65 usec
PL1             0.00 dB
PL1W       23.53637505 W
SFO1        500.1330885 MHz

F2 - Processing parameters
SI              32768
SF         500.1300000 MHz
WDW               EM
SSB                0
LB              0.30 Hz
GB                 0
PC              1.00
```

D – E10




```
Current Data Parameters
NAME          Feb11-2014
EXPNO                 12
PROCNO                 1

F2 - Acquisition Parameters
Date_         20140211
Time            22.13
INSTRUM          spect
PROBHD   5 mm PABBO BB-
PULPROG          zg30
TD              32768
SOLVENT          DMSO
NS                 32
DS                  2
SWH         10330.578 Hz
FIDRES       0.315264 Hz
AQ          1.5860212 sec
RG                203
DW             48.400 usec
DE              6.50 usec
TE              300.1 K
D1          1.00000000 sec
TD0                1

======== CHANNEL f1 ========
NUC1               1H
P1             10.65 usec
PL1             0.00 dB
PL1W       23.53637505 W
SFO1        500.1330885 MHz

F2 - Processing parameters
SI              32768
SF         500.1300000 MHz
WDW               EM
SSB                0
LB              0.30 Hz
GB                 0
PC              1.00
```

PHARMACOLOGICAL EVALUATION

8. PHARMACOLOGICAL EVALUATION

8.1 INVITRO ANTIOXIDANT ACTIVITY[54]

DPPH method

The free radical scavenging activity of the synthesized compound is evaluated by assessing their ability to reduce the colour of DPPH in chloroform. DPPH stable free radical method is an easy, rapid sensitive way to survey the antioxidant activity of specific compound.

Principle

A simple method has been developed to determine the antioxidant activity of synthesized compounds utilizes the stable 2, 2-diphenyl -1-picrylhydrazyl (DPPH) radical. The odd electron in the DPPH free radical gives a strong absorption maximum at 517nm and purple in colour. The colour turns from purple to yellow as the molar absorptivity of the DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduce DPPH-H. The resulting decolourization is stiochiometric with respect to number of electron captured.

$$DPPH + AH \longrightarrow DPPH - H + ^{\cdot}$$

Instrument

Shimadzu UV Visible spectrometer, Model 1800.

Reagent

0.1Mm Diphenyl Picryl Hydrazyl Radical in chloroform.

Procedure

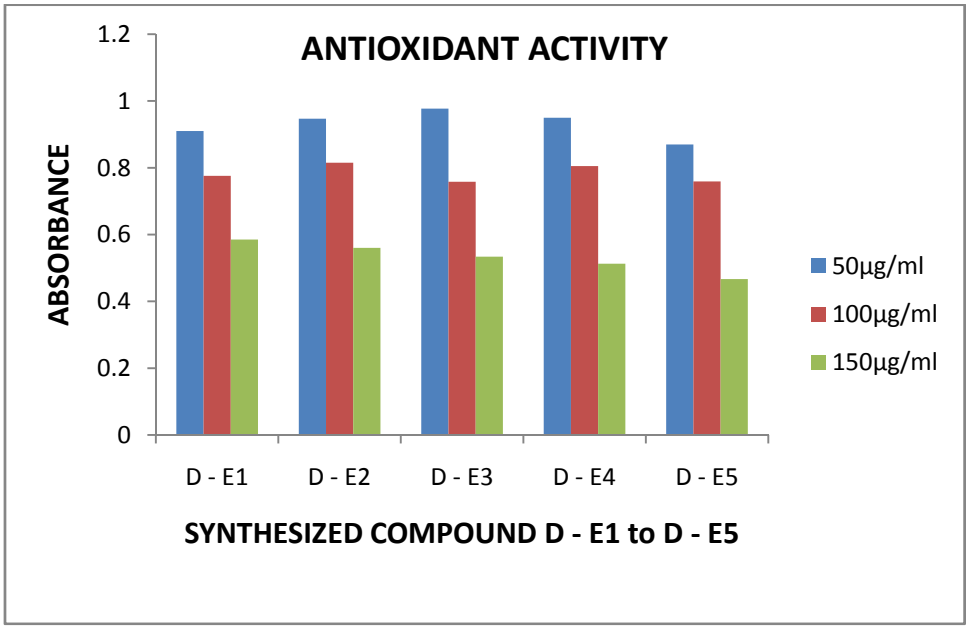
A stock solution of 1mg/ml concentration of synthesized compound was prepared. To the 1ml of various concentrations of test samples, 4ml of DPPH solution was added. Control was prepared without sample in an identical manner. DPPH was replaced by chloroform in case of blank. The reaction was allowed to be completed in the dark for about 30min. Then the absorbance was measured at 517nm. The percentage scavenging was calculated using the formula [(Control-Test)/Control]/100.

ANTIOXIDANT ACTIVITY OF SYNTHESIZED COMPOUNDS

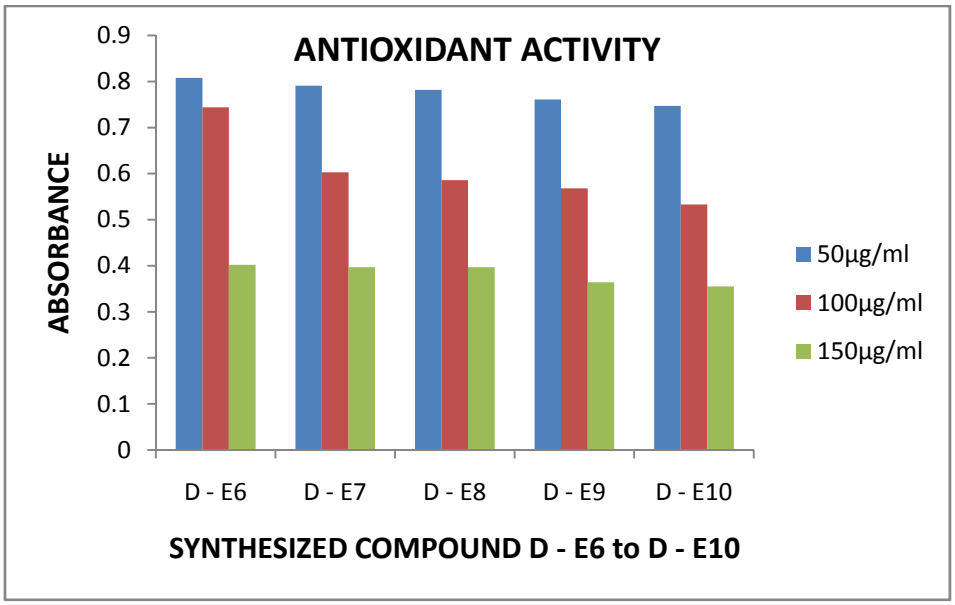
Table No: 11

COMPOUND CODE	ABSORBANCE OF DIFFERENT CONCENTRATION		
	50µg/ml	100µg/ml	150µg/ml
D – E1	0.910±0.0095	0.776±0.0049	0.585±0.0083
D – E2	0.947±0.0066	0.815±0.0052	0.560±0.0092
D – E3	0.747±0.0069	0.533±0.0052	0.355±0.0050
D – E4	0.950±0.0084	0.805±0.0080	0.513±0.0053
D – E5	0.870±0.0037	0.759±0.0025	0.467±0.0049
D – E6	0.808±0.0083	0.744±0.0078	0.402±0.0083
D – E7	0.791±0.0063	0.603±0.0087	0.397±0.0060
D – E8	0.782±0.0038	0.586±0.0036	0.383±0.0084
D – E9	0.761±0.0049	0.568±0.0073	0.364±0.0043
D – E10	0.977±0.0069	0.758±0.0060	0.534±0.0034
Standard	1.288±0.0221	0.829±0.0094	0.516±0.0043

ANTIOXIDANT ACTIVITY OF COMPOUNDS D – E1 TO D – E5



ANTIOXIDANT ACTIVITY OF COMPOUNDS D – E6 TO D – E10



8.2 INVITRO ANTICANCER ACTIVITY[54, 55]

Cell line

The human osteosarcoma cell line (MG 63) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT assay

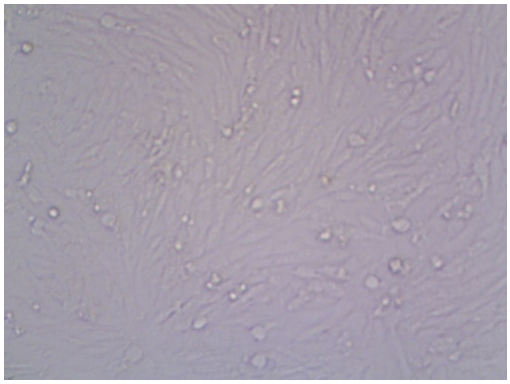
3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore,the amount of formazan produced is directly proportional to the number of viable cells.

After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The percentage cell viability was then calculated with respect to control as follows

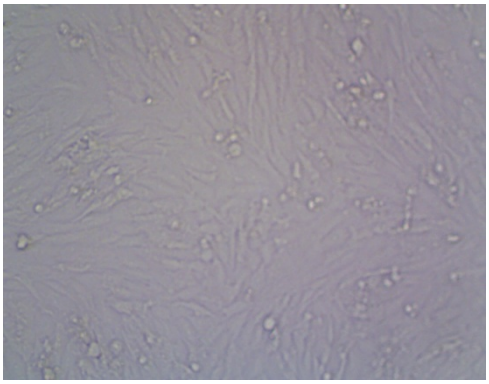
% Cell viability = [A] Test / [A]control x 100

INVITRO ANTICANCFR ACTIVITY (COMPOUND D – E3)

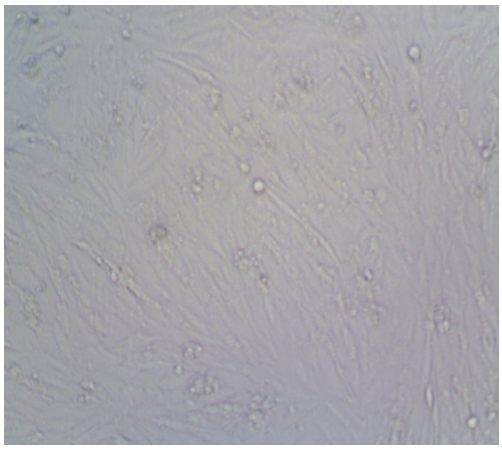
Control



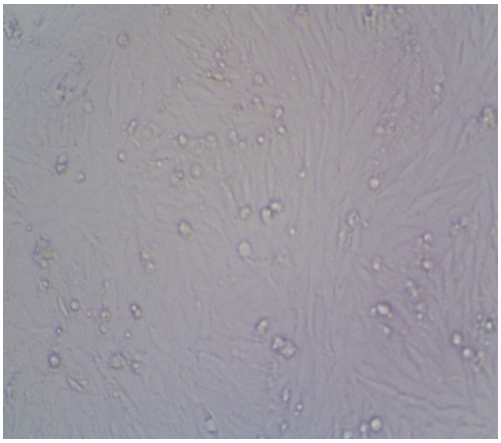
0.1µgM



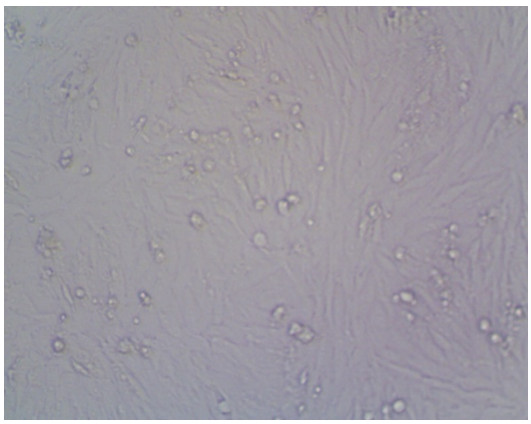
1.0µM



10µM



50µM



100µM

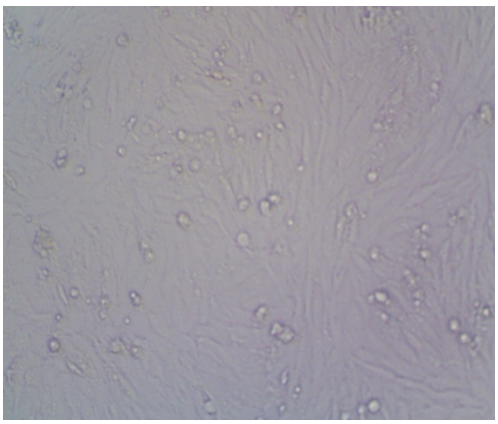
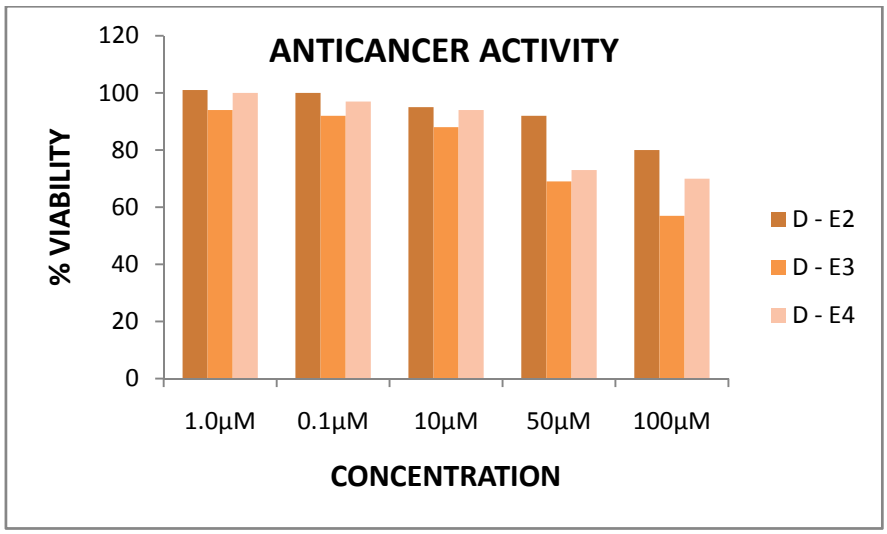


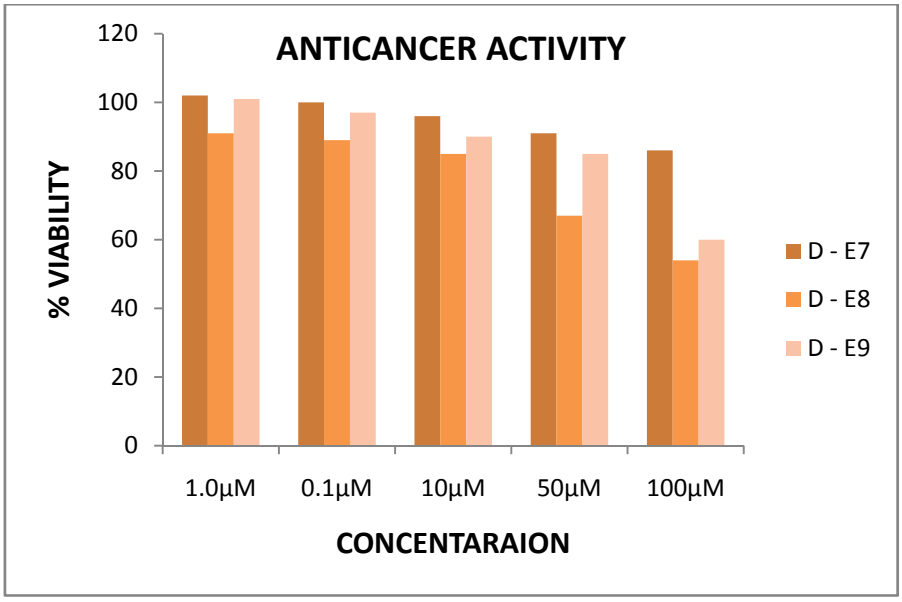
Table No: 12

COMPOUND CODE	CONCENTRATION(µM)	%CELL VIABILITY
D – E2	0.1 1 10 50 100	101 100 95 92 80
D – E3	0.1 1 10 50 100	94 92 88 69 54
D – E4	0.1 1 10 50 100	100 97 94 73 70
D – E7	0.1 1 10 50 100	102 100 96 91 86
D – E8	0.1 1 10 50 100	91 89 85 67 57
D – E9	0.1 1 10 50 100	101 97 90 85 60

ANTICANCER ACTIVITY OF COMPOUNDS D-E2, D-E3, D-E4



ANTICANCER ACTIVITY OF COMPOUNDS D-E7, D-E8, D-E9



10.3. ANTIBACTERIAL ACTIVITY[6, 27]

The microbial assay is based upon a comparison of the inhibition of growth of microorganism.

Antimicrobial activity of various synthesized compounds was studied by the presence of zone of inhibition.

- The antibacterial activities of the synthesized compounds were studied by disc diffusion method.
- All the compounds were used in the concentration of 150µg/ml, 300µg/ml using a solvent DMSO.

Details of micro organisms

Table No: 13

S.NO	ORGANISM	Gram+Ve /Gram –Ve
1	E.Coli	-Ve
2	Staphyloccus epidermidis	+Ve
3	Streptococcus pyogenes	-Ve

SOLVENT USED

DMSO

STANDARD USED

Ofloxacin in the concentration of 30µg/ml.

PREPARATION OF MULLER HINTON AGAR

Composition

Beef extract	- 10 g
Casein acid hydrosylate	- 17.5 g
Starch	- 1.5 g
Agar	- 20 g
Water	- 1000 ml

Procedure

The constituents are dissolved in distilled water and the pH was adjusted to 7.2, then the medium was sterilized in an autoclave at 121°C for 15 minutes and it was used for the bacterial inoculation.

ANTIBACTERIAL ACTIVITY (By disc diffusion method)

Muller-Hinton agar medium was prepared and transferred into sterile petriplates aseptically with the thickness of 5 – 6mm.The plates were allowed to dry at room temperature and were inverted to prevent condensate falling on the agar surface. Uniform thickness of the medium was obtained by placing the plates on leveled surface.

Standardized bacterial inoculums were applied to the plates and spread uniformly over the surface of the medium by using a sterile non – absorbent cotton swap and finally the swap was passed around the edge of the medium. The inoculated plates were closed with the lid and allow drying at room temperature.

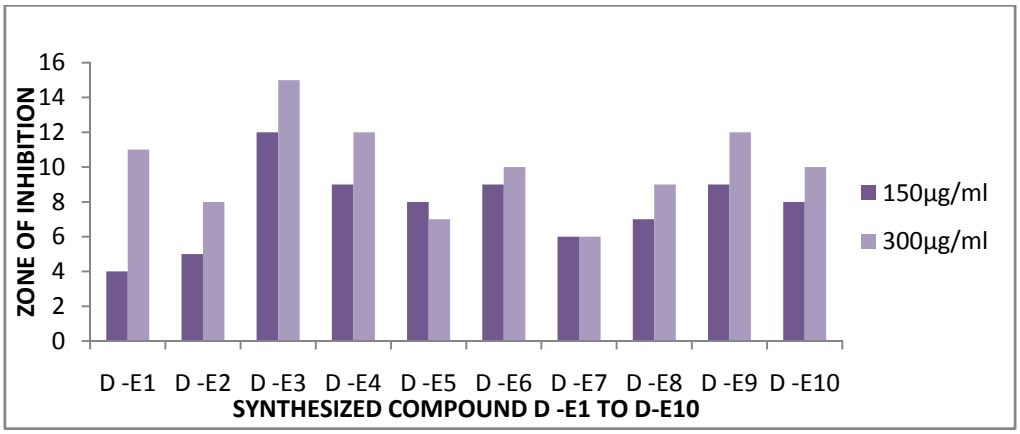
The sample impregnated discs were placed on the inoculated agar medium. All petriplates were incubated at 37°C for 24 hours. After incubation, diameter of zone of inhibition produced by the sample was measured and reading observed in millimeter.

ANTIBACTERIAL ACTIVITY AGAINST BACTERIA

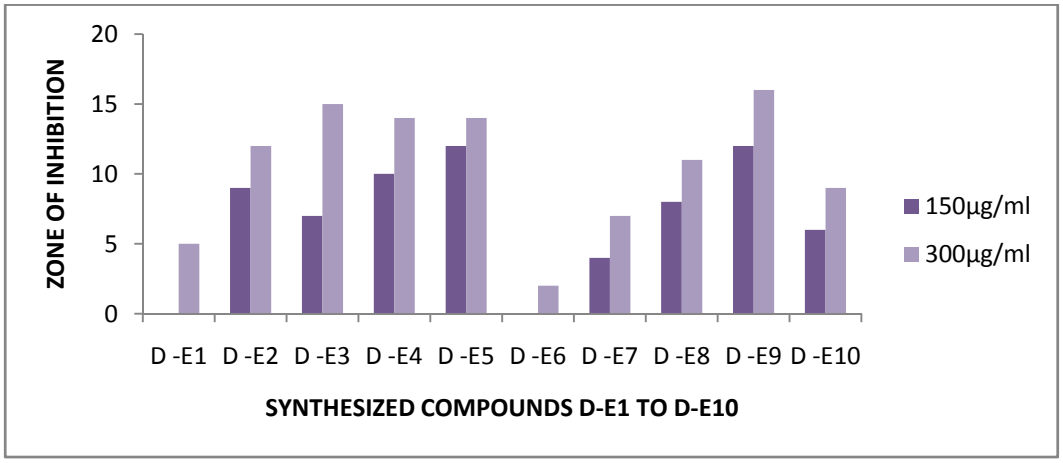
Table No: 14

COMPOUND CODE	ZONE OF INHIBITION IN MM					
	E.COLI		STAPH.EPIDERMIDIS		STREP.PYOGENES	
	150µg/ml	300µg/ml	150µg/ml	300µg/ml	150µg/ml	300µg/ml
D – E1	4	11	R	5	12	12
D – E2	5	8	9	12	4	7
D – E3	12	15	7	15	11	14
D – E4	9	12	10	14	8	16
D – E5	8	7	12	14	R	6
D – E6	9	10	R	2	9	11
D – E7	6	6	4	7	6	9
D – E8	7	9	8	11	8	14
D – E9	9	12	12	16	7	13
D – E10	8	10	6	9	4	10
CONTROL	R	R	R	R	R	R
STANDARD	17		18		18	

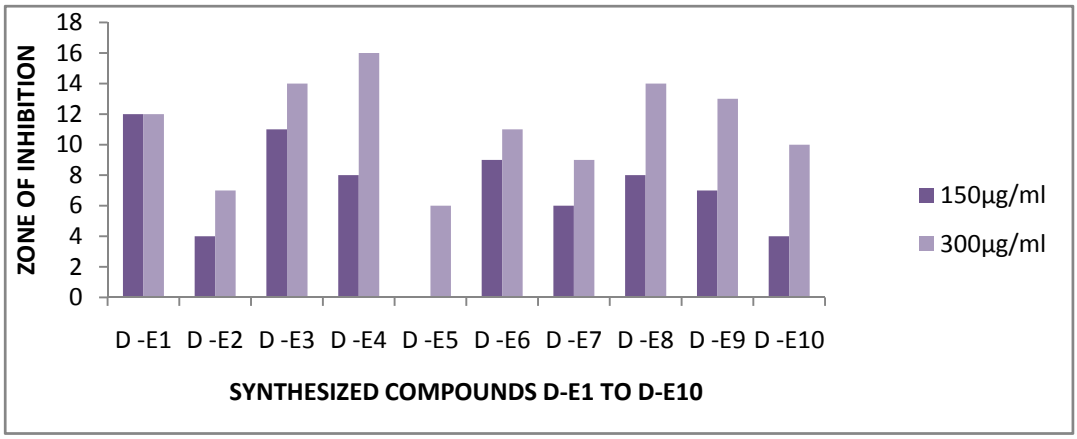
ANTIBACTERIAL ACTIVITY AGAINST E.COLI



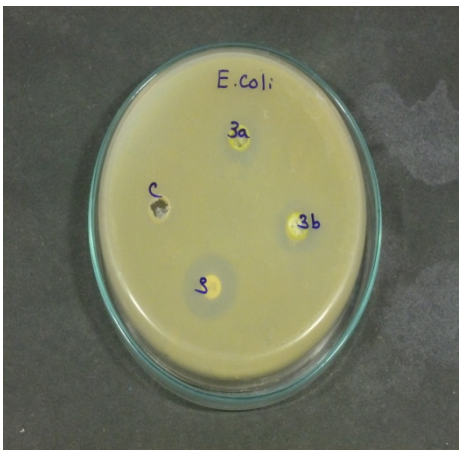
ANTIBACTERIAL ACTIVITY AGAINST STAPH.EPIDERMIS

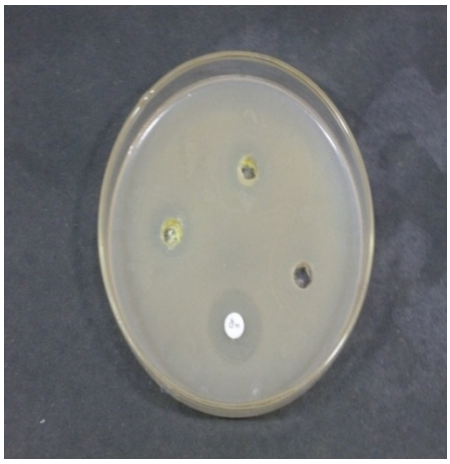


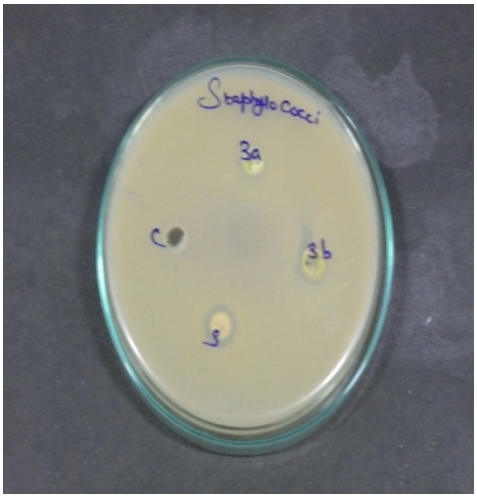
ANTIBACTERIAL ACTIVITY AGAINST STREP.PYOGENES

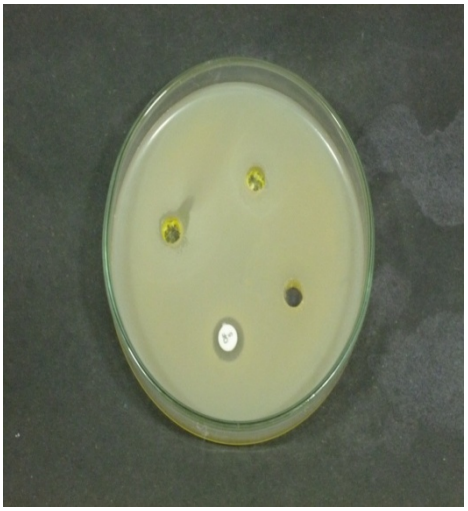


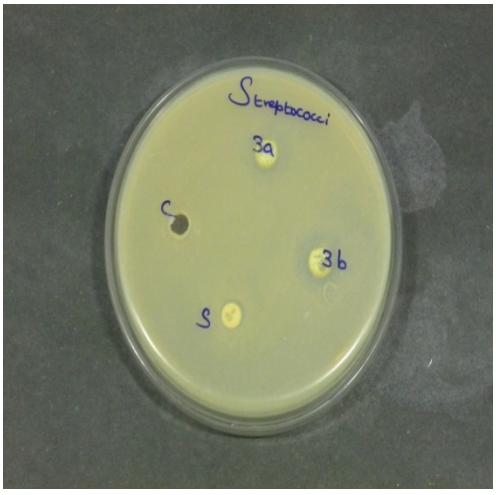
ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS D-E3, D-E4, D-E8

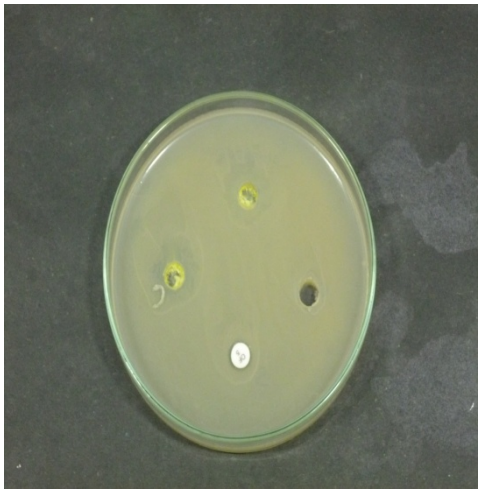












8.4. ANTIFUNGAL ACTIVITY[9, 27]

The microbial assay is based upon a comparison of the inhibition of growth of microorganism.

Antifungal activity of various synthesized compounds was studied by the presence of zone of inhibition.

- The antifungal activitiy of the synthesized compounds were studied by disc diffusion method.
- All the compounds were used in the concentration of 150µg/ml, 300µg/ml using a solvent DMSO.

Details of micro organism

Table No: 15

S. NO	ORGANISM
1	Candida albicans
2	Aspergillus parasiticus

SOLVENT USED

DMSO

STANDARD

Fluconazole in the concencentration 30µg/ml.

PREPARATION OF POTATO DEXTROSE AGAR MEDIUM

Composition

Potato	- 200g
Dextrose	- 20g
Agar	- 20g
Water	- 1000ml

Procedure

Scrub but do not peel the potatoes and cut into 12mm cubes. Boil 200g potato in 1litre of water for 60 minutes. Squeeze as much of the pulp as possible through a fine sieve. Add agar and boil till dissolved. Add dextrose and make up to 1litre. Dispense in requiredamounts taking care to keep solids in suspension. Autoclave at 115°C and pour approximately 20ml amounts into petri dishes.

ANTIFUNGAL ACTIVITY (By disc diffusion method)

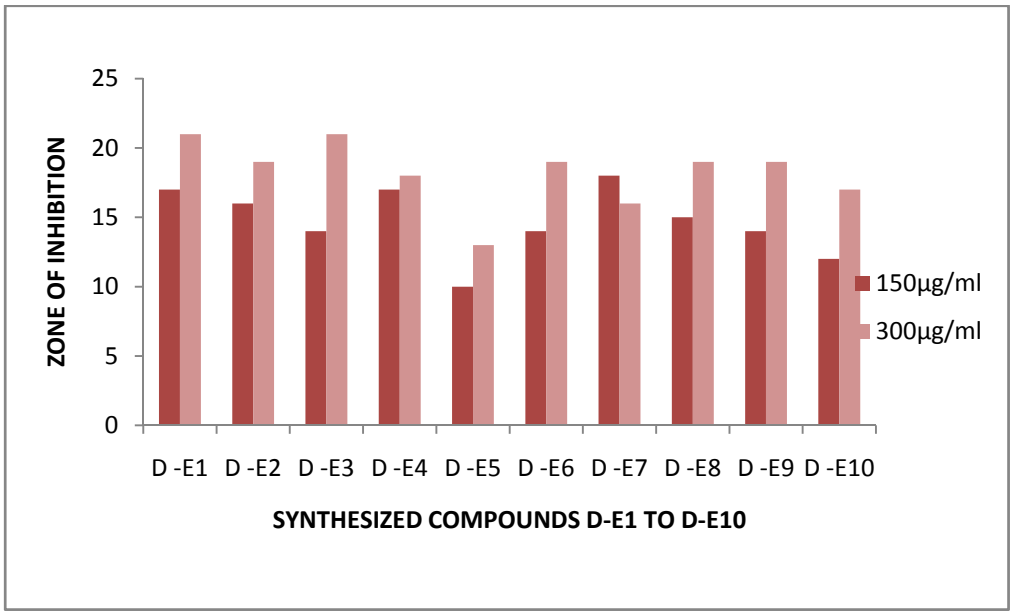
The plates were inoculated by dipping a sterile swab into inoculums. The inoculation was dried at room temperature in aseptic condition. Ditch the bore in plate, to this bore add prepared antibacterial solution. These plates were placed in an incubator at 22°C within a few minutes of preparation. After 7 days of incubation the diameter of zone of inhibition was measured and reading observed in millimeter.

ANTIFUNGAL ACTIVITY AGAINST FUNGI

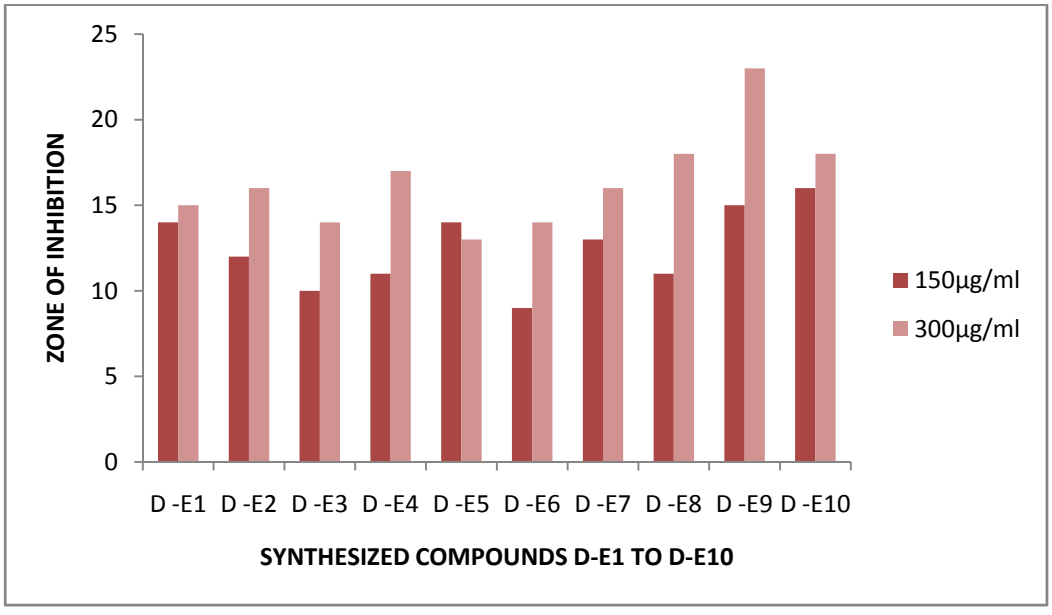
Table No: 16

COMPOUND CODE	ZONE OF INHIBITION IN MM			
	CANDIDA ALBICANS		ASPERGILLUSPARACITICUS	
	150µg/ml	300µg/ml	150µg/ml	300µg/ml
D – E1	17	21	14	15
D – E2	16	19	12	16
D – E3	14	21	10	14
D – E4	17	18	11	17
D – E5	10	13	14	13
D –E6	14	19	9	14
D – E7	18	16	13	16
D – E8	15	19	11	18
D – E9	14	19	15	23
D – E10	12	17	16	18
CONTROL	R	R	R	R
STANDARD	20		17	

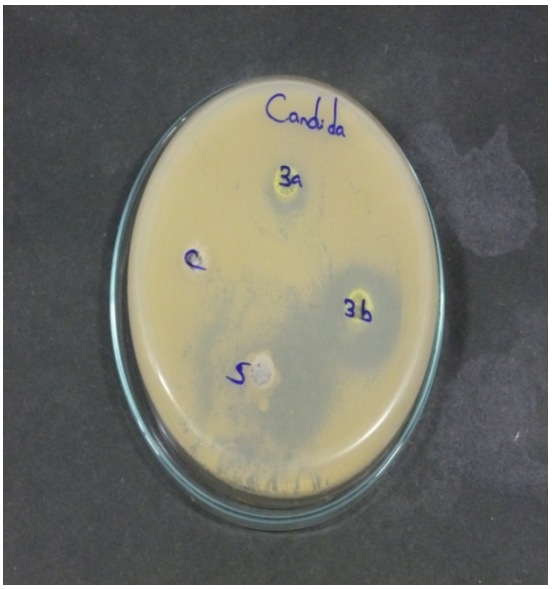
ANTIFUNGAL ACTIVITY AGAINST CANDIDA ALBICANS

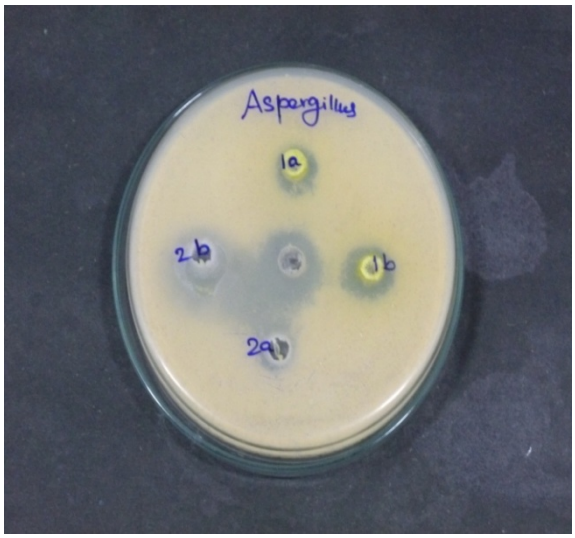


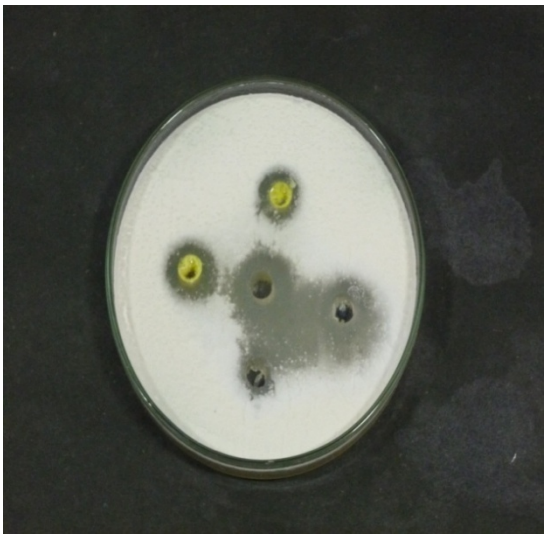
ANTIFUNGAL ACTIVITY AGAINST ASPERGILLUS PARASITICUS



ANTIFUNGAL ACTIVITY OF SYNTHESIZED COMPOUND D-E4, D-E8, E-E9







RESULT AND DISCUSSION

9. RESULT AND DISCUSSION

- In this present study, the molecular designing of the compounds were carried out by using different software.
- The lipinkis rule of five was calculated by chemdoodle software and results were shown in **Table No:1**
- The Molecular formula, Molecular weight and IUPAC name were predicted and shown in **Table No: 2 & 3.**
- The compounds were synthesized by *"Leuckart reaction"* which shows good percentage yield, melting point and solubility of the compounds were determined and shown in **Table No: 4 & 6.**
- The compounds are monitored by TLC and R_f value were calculated and shown in **Table No:7**
- Elemental composition were found and calculated in percentage and results obtained were shown in **Table No: 5**
- The compounds were confirmed by spectral analytical data.
- The results for IR spectra are shown in **Table No: 8**
- The results for NMR spectra are shown in **Table No: 9**
- The results for MASS spectra are shown in **Table No: 10**
- All the ten synthesized compounds were screened for their antioxidant, anticancer, antibacterial, antifungal activity.
- The antioxidant activity was performed by DPPH method and results obtained were showed in **Table No: 11**
- The anticancer activity (osteosarcoma) was performed by MTT assay method and results obtained were shown in **Table No:12**

- The antibacterial activity was performed against E. coli, Staphylococcus epidermis, Streptococcus pyogenes organism were shown in **Table No:13**
- The zone of inhibition was performed by disc diffusion method. The results were measured in millimeter and shown in **Table No:14**
- The graphical representation of all the ten compounds were shown and compared with standard drug (Ofloxacin).
- The antifungal activity was performed against Candida albicans and Aspergillus parasiticus were shown in **Table No:15**
- The zone of inhibition was performed by disc diffusion method. The results were measured in millimeter and shown in **Table No:16**
- The graphical representation of all the ten compounds were shown and compared with standard drug (Fluconazole).

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

- ✓ Preliminary screening of the 1-substituted tetrahydropyrimidine derivatives was done by using molinspiration, chemdoodle and chemsketch software.
- ✓ The present study describes the synthesis of 1-substituted tetrahydropyrimidine derivatives by Leuckart reaction. This methodology offers the spirited advantages having lesser time reaction and yield higher percentage of products.
- ✓ Melting point was found for the synthesized compound and purity of the synthesized compound was analyzed by TLC.
- ✓ The structures of the synthesized compounds were elucidated by IR, NMR and Mass spectroscopy.
- ✓ The synthesized compounds were screened for anti oxidant, anticancer, antibacterial, antifungal activity.
- ✓ The in-vitro anti oxidant property for all the compounds showed positive results.

  The compounds D – E2, D – E3, D – E4, D – E7, D – E8, D – E9 showed more potent activity. These six compounds were selected and evaluated for anticancer activity. The results obtained showed that synthesized compounds D – E3, D – E8, showed anticancer activity against cancer cells.
- ✓ It proves the suitable structural modification will have to be carried to get novel compound having potent anticancer activity with least effect on normal cells.
- ✓ The antimicrobial activities of synthesized compounds were to obtained zone of inhibition by disc diffusion method.

- ✓ P-dimethylamino benzaldehyde and cinnamaldehyde based tetrahydropyrimidine derivatives were react with acetophenone and benzophenone which gives D – E3, D – E4 and D – E8, D – E9 respectively. These four compounds exibit best antibacterial activity as compared to standard drug (Ofloxacin).
- ✓ Among the synthesized compounds were found to be good antifungal activity as compared to standard drug (Flucconazole).
- ✓ Furthermore biological activities such as anticonvulsant, anti-inflammatory, antimalarial, antitubercular activities can be done for the synthesized compound in the future.

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