Synthesis of Pyrimidine Derivatives and Investigation of their Antimicrobial Activity

by

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemistry

Khulna University of Engineering & Technology
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May, 2017

DADICATED

TO MY BELOVED

PARENTS

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May, 2017 Reshma parvin

Abstract

The research work was involved in an efficient procedure for the attachment of 4- amino acetophenone with benzaldehyde in the presence of 10% NaOH to produce 1-(4-Amino-phenyl)-3-phenyl-propenone **(I)** or arylidine acetophenone. 4-{1-[(2,4-Dinitro-phenyl)-hydrazono]-3-phenyl-allyl}-phenylamine **(II)** has been synthesized by the reaction of 2,4 DNP with 1-(4-Amino-phenyl)-3-phenyl-propenone. Reaction of barbituric acid with arylidine acetophenone under refluxing condensation using 50% aqueous ethanol which on further reflux to give the corresponding pyrimidine derivatives, 7-(4-Amino-phenyl)-5-phenyl-1, 5-dihydro-pyrano [2, 3-d] pyrimidine-2, 4-Dione **(III)**. The structures of the synthesized compounds were characterized by their IR, NMR spectral data. The antibacterial and antifungal activity of the compounds was also investigated. The antimicrobial activity of the compounds at concentration at 300µg/disc was performed against three gram-positive bacteria (*Bacillus cereus, Staphylococcus aureus* and *Listeria monocytogenes*) and three gram-negative bacteria (*Escherichia coli, Salmonella typhimurium, and citobacteria frundi*) by Kirby-Bauer disc diffusion method using Ciprofloxacin as standard. Antifungal screening was carried out against one fungi (*Tricodarma harzianum*) using Michonazole as standard. Compound **I**, 1-(4-Amino-phenyl)-3-phenyl-propenone exhibits no activity against the tested bacterial strains but showed moderate activity against the tested fungi *Trichoderma harziniaum* having inhibition zone 11 mm. Compound **III**, 7-(4-Amino-phenyl)-5-phenyl-1,5-dihydro-pyrano[2,3-d] pyrimidine-2,4-Dione exhibits broad spectrum activity against both gram negative and gram positive strains tested except *Listeria monocytogenes.* Also the compound **II**, 4-{1-[(2,4-Dinitro-phenyl)-hydrazono]-3-phenyl-allyl}-phenylamine showed promising antibacterial activity having inhibition zone of 24 mm against *Ctirobacteria freundi* having no antifungal activity. Those bacteria revealed the zone of inhibition were 11-24 mm. The presence of an amino and unsaturated ketone function in synthesized compound may be responsible for their antibacterial and antifungal activity.

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Abbreviation

UV	Ultraviolet
IR	Infra-red
NMR	Nuclear magnetic resonance
s	Singlet
Bs	Broad single
d	Doublet
J	Coupling constant
TLC	Thin Layer Chromatography
R_f	Retarding factor
Bp	Boiling point
Hz	Hertz
δ	Chemical shift
TMS	Tetra methyl Silane
DMSO	Dimethysulfoxide

CHAPTER I

INTRODUCTION

CHAPTER I

Introduction

1.1 History of Chalcone

There is growing interest in the pharmacological potential of natural products is chalcones constitute an important group of natural products [1]. The chemistry of chalcones has generated intensive scientific studies throughout the world. Especially interest has been focused on the synthesis and biodynamic activities of chalcones. The name "Chalcones" was given by Kostanecki and Tambor [2]. Chalcone is an aromatic ketone that forms the central core for the variety of important biological compounds, which are collectively known as chalcones. These compounds are also known as benzalacetophenone or benzylidene acetophenone. In chalcones, two aromatic rings are linked by an aliphatic three carbon chain. Chalcones are 1,3-diphenyl-2-propene- 1-one, member of flavonoids family, are important class of natural or synthetic products in which two aromatic rings are linked by a three carbon α, β- unsaturated carbonyl system (Figure 1.1) [3].

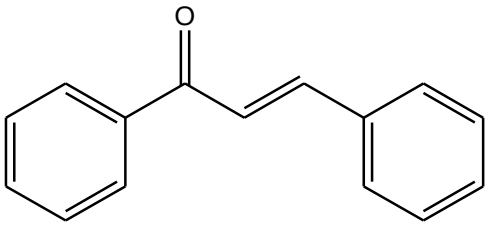


Figure 1.1: Chalcone

Rings are interconnected by a highly electrophilic three carbon α, β-unsaturated carbonyl system that assumes linear or nearly planar structure [4]. They contain the ketoethylenic group (–CO–CH=CH-). These are coloured compounds because of the presence of the chromophore -CO-CH=CH-, which depends in the presence of other auxochromes [2]. Chalcones possess conjugated double bonds and a completely delocalized π-electron system on both benzene rings. The energy minimized 3D structure of chalcone has been shown in the (Figure 1.2).

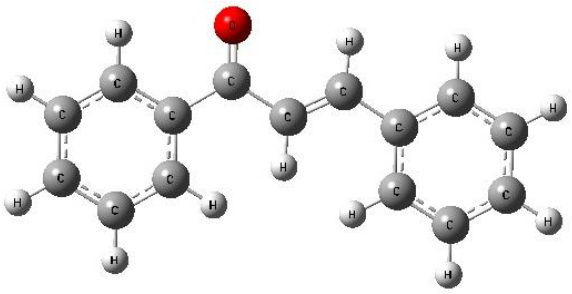


Figure 1.2: 3D structure of chalcone

Chalcones have crystal structure. The dihedral angle between the two phenyl rings is 13.0(1)°, and the dihedral angle from the plane of C7/C8/C9 to the phenyl rings (C1 to C6 and C10 to C15) are 13.8(1)° and 2.6(1)°,respectively, indicating that the central C7-C8-C9 fragment lies nearly in the phenyl ring plane of C10 to C15, but rather more displaced out of the other benzene ring of C1 to C6 show in (figure 1.3) below. The molecule forms a zigzag chain by C-H···π (arene) hydrogen bonds along the *c* axis. There also exist intermolecular hydrogen bonding interactions involving C11 acting as H-bond donor, via H11, to O in the adjacent molecules at -*x*,1-*y*,1-*z*, resulting in a three-dimensional network [5].

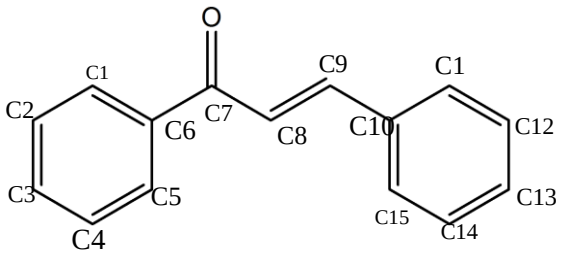


Figure 1.3: The crystal structure of chalcone

Chalcones are natural compounds that are largely distributed in plants, fruits, and vegetables. They belong to the flavonoid group of molecules and some of them exhibit numerous biological activities. They are precursors in flavonoid biosynthesis. The enzymatic cyclization of the 6-hydroxychalcones leads to the formation of flavanones and subsequently to a large number of flavonoid groups including flavones, flavonols, dihydroflavonols, aurones and isoflavones [6].

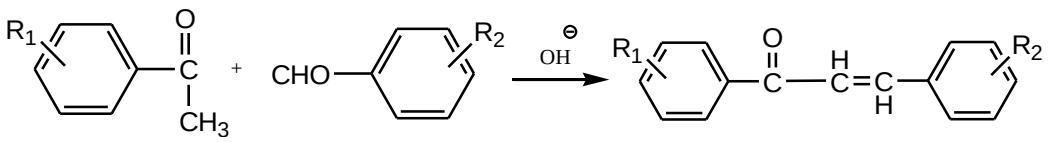
Chalchones are one of the major classes of naturally occurring compounds. A large number of synthetic routes have been reported for the synthesis of chalchones, the most classical and general being the Claisen-Schmidt condensation. Chalchones and their derivatives have a huge importance in medicinal chemistry [7].

Chalcones are the precursors in the biosynthesis of anthocyanins and flavones. This is due to some specific advantages of the reaction of chalcones synthesis which are as follows [8, 12].

- Solvent free reaction
- Use of non-hazardous chemicals
- Quick reaction
- High yield
- Minimum energy requirement
- Room temperature reaction

1.1.1 Chemistry of Chalcone

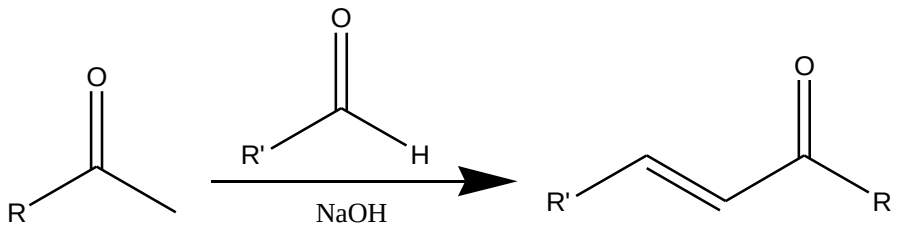
Al varieties of techniques are accessible for the synthesis of chalcone. The greatest appropriate method is the one that includes the Claisen-Schmidt condensa tion of equimolar amounts of a substituted acetophenone with substituted benzaldehyde in the presence of an alcoholic alkali. In the Claisen- Schmidt reaction the concentration of an alkali used usually ranges between 10-60%.The reaction is carried out at about 50° C for 12-15hours or at room temperature for one week.



Scheme: 1.1

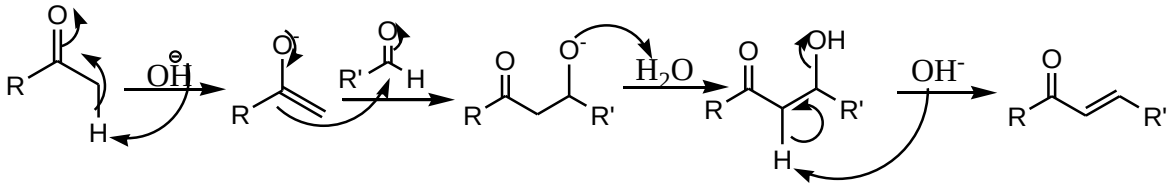
Claisen -Schmidt condensation

Their action between a ketone and a carbonyl compound lacking an alpha -Hydrogen (Cross Aldol condensation) is called Claisen -Schmidt condensation reaction [9].



Scheme: 1.2

Mechanism of Claisen -Schmidt condensation



enolate aldol product product

Scheme: 1.3

Chalcones are used to synthesize several derivatives like cyanopyridines, pyrazolines isoxazoles and pyrimidines having different heterocyclic ring systems. Different methods are available for the preparation of chalcones. Chalcones and substituted chalcones can be synthesized in laboratory by Claisen-Schmidt condensation of acetophenone or substituted acetophenones with aldehydes [10]. Chalcone bears a very good synthon so that variety of novel heterocyclic with good pharmaceutical profile can be designed. In recent years there has been a lot of work done in preparation of chalcones containing heterocyclic.

1.1.2 Biological and Pharmacological activities of Chalcone

Chalcones have been used as intermediates for the preparation of compounds having therapeutic value. Many reviews reveal that chalcone derivatives exhibit diverse pharmacological activities, such as potential cytotoxic agents, antimicrobial agents, antiviral, anti-inflammatory, anesthetic, and etc. Chalcones either natural or synthetic and their heterocyclic are known to exhibit various biological activities. They have been reported to possess antioxidant [11], antimicrobial [1, 12-14], antileishmanial [13], anti-inflammatory, antitumour [15] and antibacterial activity cytotoxic activity [16]. The presence of a reactive, unsaturated keto function in chalcones is found to be responsible for their antimicrobial activity, which may be altered depending on the type and position of substituent on the rings [17]. The present works was designed to synthesize hetrocyclic compounds via chalcones route and were evaluated by different physical properties, Spectral analysis and antibacterial activity. In view of the variety of pharmacological properties exhibited by chalcones and pyrimidines, it was planned to synthesize new series of chalcones, and pyrimidine derivatives.

1.2 Pyrimidine Derivatives

Chalcone are natural biocides and are well known intermediates for synthesizing heterocyclic compounds [18]. Heterocyclic rings have played an important role in medicinal chemistry, serving as key templates central to the development of numerous important therapeutic agents [19]. Pyrimidines can be regarded as a cyclic amine and also be known as m-diazone (or) 1, 3-diazine (Figure 1.4). At very early period in the history of organic chemistry, pyrimidines (“m-Diazine”) were known as the breakdown products of uric acid. Pyrimidine is the most important member of all the diazines as this ring occurs widely in living organisms [20].

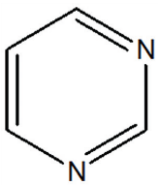


Figure 1.4: Pyrimidine derivatives

The first pyrimidine derivative to be isolated was alloxan in 1818 by Brugnatelli, oxidizing uric acid with nitric acid [21]. The name pyrimidine (combination of words pyridine and amidine) was first applied by Pinner. Pyrimidines (Fig. 1) are the heterocyclic aromatic compounds similar to benzene and pyridine containing two nitrogen atoms at positions 1 and 3 of the six-membered rings (Fig 01). Pyrimidine having molecular formula of $C_4H_4N_2$ and molecular weight = 80. It is isomeric with two other forms of diazene [22].

It is the parent substance of large group of heterocyclic compounds & plays a vital role in many biological processes as found in nucleic acids, several vitamins, co-enzymes and purines. In fact, there are seven types of pyrimidine ring closure (i-vii) depending upon the nature of fragments that combine together to form the pyrimidine nucleus [23]. Also, pyrimidine derivatives can be synthesized by ring transformation and ring expansion.

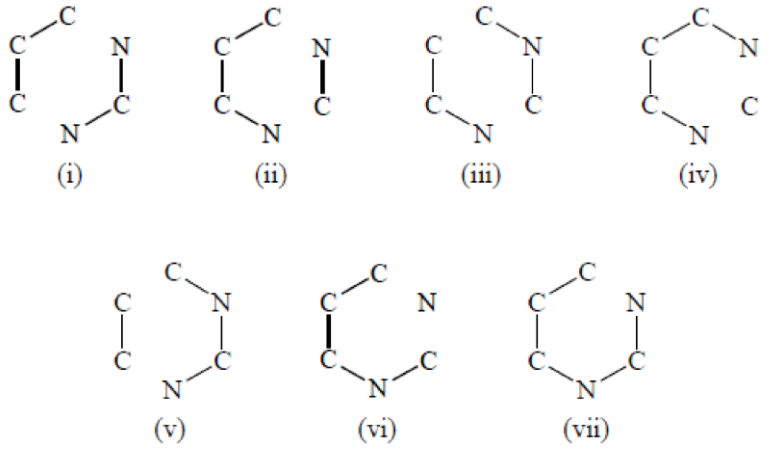
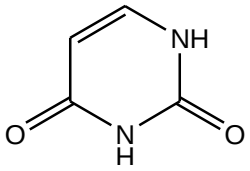


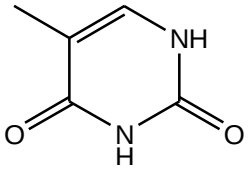
Figure 1.5: Classification of pyrimidine ring

Pyrimidine itself is not found in nature but substituted pyrimidines and compounds containing the Pyrimidine ring are widely distributed in nature. In addition, pyrimidine and fused pyrimidine derivatives are one of the most prominent structures found in nucleic acid including uracil, thymine, cytosine, adenine, and guanine are fundamental building blocks for deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) [24].



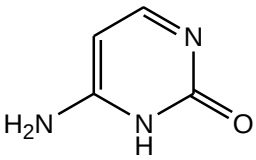
1

Uracil



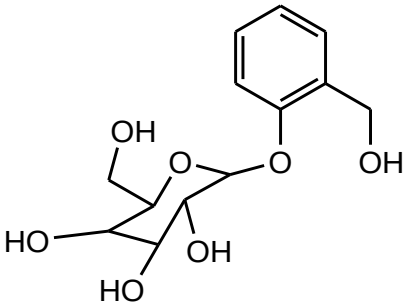
2

Thymine



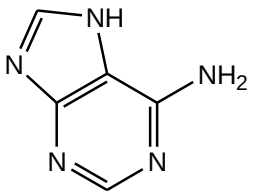
3

Cytosine



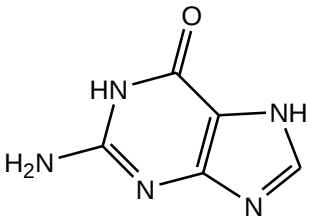
4

Glycosides



5

Adenine



6

Guanine

Figure 1.6: Derivatives of pyrimidine

The nomenclature of pyrimidines is straight forward. However, like other heterocyclics, tautomeric hydroxyl groups yield complications since they exist primarily in the cyclic amide form. For example, 2-hydroxypyrimidine is more properly named 2-pyrimidone. A partial list of trivial names of various pyrimidines exists [25].Several (mainly uracil, thymine, and cytosine (Figure 1.6). Such as

pyrimidines have been isolated from the nucleic acid hydrolyses. The nucleic acid are essential constituent of all cells and thus all living matter cytosine is found to be present in both types of nucleic acids i.e., ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) while uracil is present only in RNA and thymine only in DNA [26]. Pyrimidine is a colourless compound, having melting point 22.5ºC and boiling point at 120ºC. Its dimensions have been determined by an X- ray diffraction study of a crystal at 2ºC and closely resemble those of pyrimidine (six membered heterocyclics with one nitrogen atom in their ring figure 1.7).

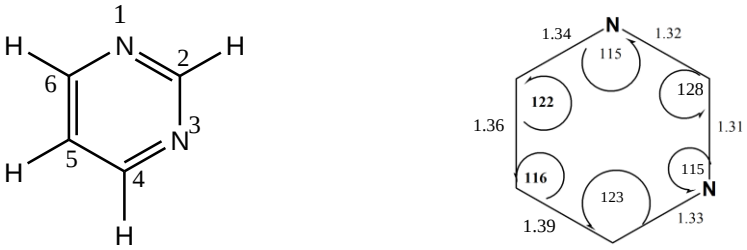


Figure 1.7: X-ray diffraction study of a pyrimidine crystal

The self- consistent π-electron densities, the carbon –carbon distance in the pyrimidine are (1.35-1.40 Aº) closely resemble those of pyrimidine which are not similar to aliphaticcarbon-carbon single bond distance (1.54 Aº). They are similar to that for benzene (1.40 Aº) or between this length and that for ethylene (1.33 Aº). The general similarity of the size and shape of the pyrimidine of that of benzene and pyridine is consistent with its highly chemical characteristics. But due to differences between the bond angles and distance of theses ring systems suggested that of pyrimidine 109 kj(kcal)/mole. Pyrimidine is best considered as a resonance hybrid to which the uncharged equivalent structures 1 and 2 and the charged structures 3-8 contribute (Figure 1.8). The self-consistent pai electone densities, calculated for ground state of pyrimidine, are 0.776, 0.825, and 1.103 for positions 2, 4, and 5, respectively.

Kekule structurs:

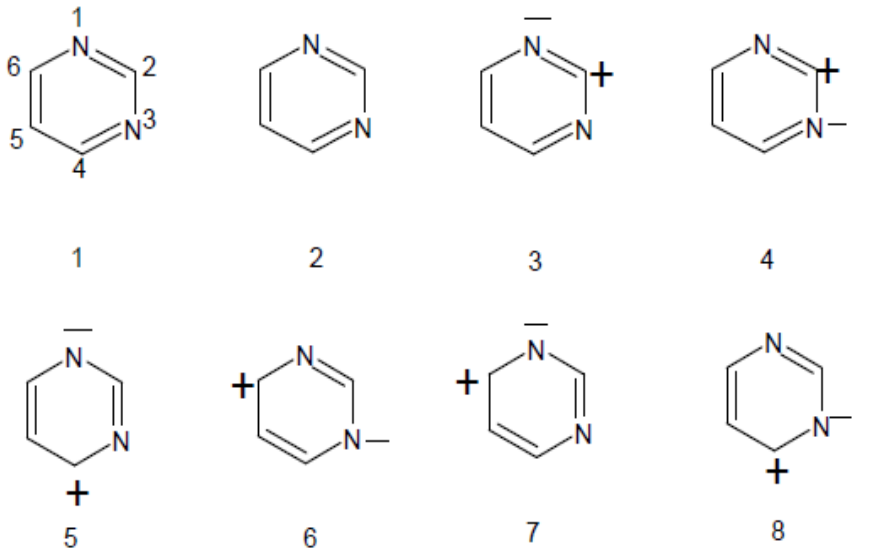


Figure 1.8: 3-8 charged structures

On the other hand, heterocyclic compounds occur very widely in nature and are essential to life. Nitrogen- containing heterocyclic molecules constitutes the largest portion of chemical entities, which are part of many natural products, fine chemicals, and biologically active pharmaceuticals vital for enhancing the quality of life [27].

Synthetic studies of fused pyrimidine have been reported extensively because of their structural diversity and association with a wide spectrum of biological activity. pyrimidines are also of pharmacological interest due to their anti-inflammatory psychopharmacological bactericidal and antibacterial, anticancer, antitubercular antioxidant, anticonvulsant, antibacterial, antiplasmodial, antifungal, anticancer, [28-30], antimicrobial, antibiotics, antiviral activity as inhibitors of HIV-1 reverse transcriptase antifolates [31] and antihismatic activity [32] . Moreover, in recent years, it was reported that many fused pyrimidine analogues inhibitors of tyrosine kinase and cyclin-dependent kinases, which are involved in mediating the transmission of mitogenic signals and numerous other cellular events , including, cell proliferation, migration, differentiation, metabolism, and immune responses. It was also found that many of these derivatives may block proliferation of various cancer cell lines [33]. Based on the above observation and in

continuation of this research work, herein reported the synthesis of some pyrimidine derivatives and evaluating their antibacterial activity.

1.3 History of Barbituric acid

For almost a half of the period many derivatives were synthesized, but only early in the 20th period it was discovered that some barbituric acid derivatives have an effect on the central nervous system. During his famous studies on the constitution of uric acid, von Baeyer ' isolated a previously unknown compound to which he gave the name "barbituric acid" and showed that on cleavage it gave malonic acid and urea [34]. Barbituric acid was first synthesized November 27, 1864, by German chemist Adolf von Baeyer. This was done by condensing urea (an animal waste product) with diethyl malonate (an ester derived from the acid of apples). There are several stories about how the substance got its name. The most likely story is that Baeyer and his colleagues went to celebrate their discovery in a tavern where the town's artillery garrison were also celebrating the feast of Saint Barbara – the patron saint of artillerymen. An artillery officer is said to have christened the new substance by amalgamating Barbara with urea. Barbituric acid, chemically 2, 4, 6-trioxohexahydropyrimidine (figure 1.9), a cyclic amide used as the parent compound to produce barbiturates that act as central nervous system depressants [35].

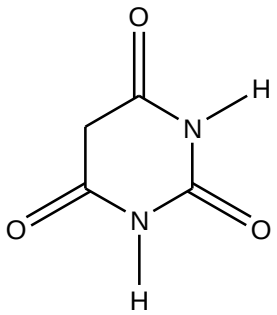


Figure 1.9: Barbituric acid

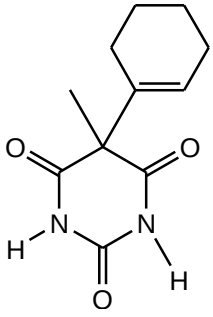
During that period many derivatives of barbituric acid were used as drugs side-effects. Barbituric acid itself does not give sedative and hypnotic effects, although they are probably most familiar as 'sleeping pills' but the substituted derivatives with alkyl or aryl group at position 5 provide effects. Also derivatives of barbituric acid, widely used in

medicines, for e.g., veronal, pentothiol, luminol are used as hyphotics while is used as anesthetic, Purines, Uric acid. Barbuturic acid and a mixture of amtimalarial and antibacterial also contain the Pyrimidine ring [36].

1.3.1 Classifications of Barbiturates according to their duration of action

Barbituric acids and their consequent derivatives are classified into four classes, according to their duration of action metabolic degradation. The extent of the effects of barbiturates as well as the protein binding attraction of barbiturates are directly proportional to the chain length of the hydrocarbon attached to the *C-5* position of the barbituric acid ring [37].For example, the classes which includes are following

1) Ultra short-acting barbiturates: Contain compounds which are metabolized rapidlyand is highly lipid soluble. Examples include hexobarbital which is C-5 substituents, thiopental is C-2 substituents have that are hydrocarbons of four or more carbon units.



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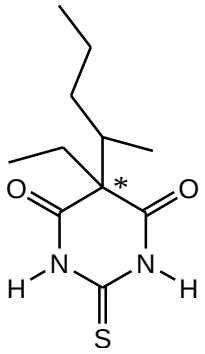
Hexobarbital



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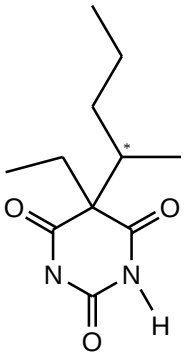
Thiopental

2) Short-acting barbiturates: Those are lipid soluble and bind to proteins. For example examples of short acting barbiturates include pentobarbital and secobarbital (C-5 substituents) etc.



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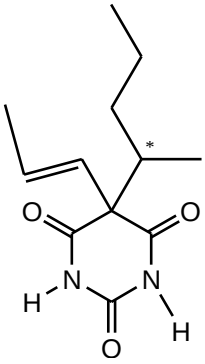
Pentobarbital



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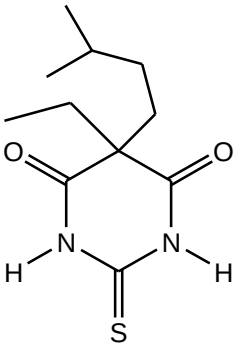
Secobarbital

3) Intermediate-acting barbiturates: These derivatives are typically used as hypnotics for persons waking in the middle of the night. Such examples butabarbital and amobarbital (C-5 subtituents).



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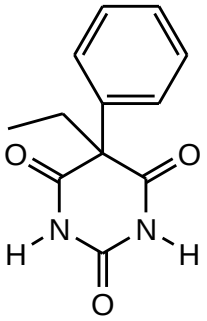
Amobarbital

4) Long acting barbiturates. These compounds use as a hypnotic effect for longer than six hours, causing sedation and subsequent drowsiness. Such examples include Phenobarbital and veronal active anti-epileptic.



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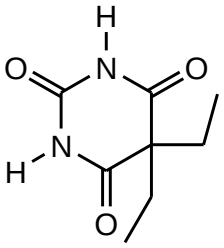
Phenobarbital



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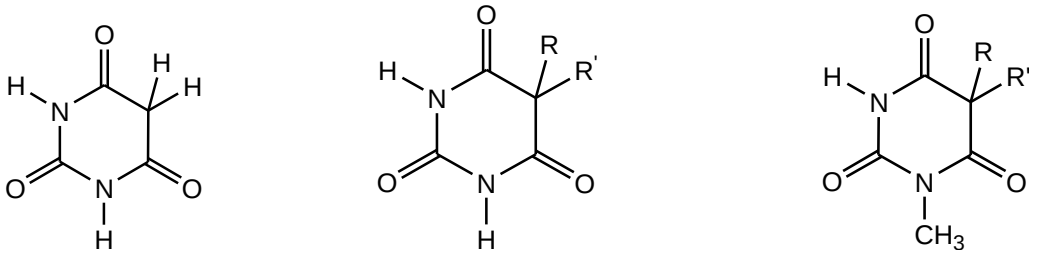
Veronal

1.3.2 Production method

It is derived by the reaction of diethyl malonate and urea. First put Urea in a reaction tank containing methanol ,heat , reflux , dissolve, then add the dried diethyl malonate and sodium methoxide, the reaction is refluxed at 66-68 ℃ for 4-5h, after distillation to recover methanol, cooling to 40-50 ℃, add dilute hydrochloric acid to adjust to pH 1-2.Cool to room temperature, throw to obtain crude, wash with distilled water once, dry to get crude , and then purify with water and activated carbon, dry to obtain products. Industrial barbituric acid is white or pink crystalline powder, strongly acidic, more than 98% content, melting point ≥245 ℃. Material consumption fixed: diethyl malonate 1098kg / t, urea 476kg / t, hydrochloric acid (reagent grade Ⅲ) 681kg / t, sodium methanol (28%) 369kg / t, methanol 1025kg / t [33].

1.3.3 Physical properties of barbituric acid

Barbituric acids and the active derivatives of barbituric acid are considered both hydrophilic, due to the 2, 4, 6-pyrimidinetrione ring system, and lipophilic, depending on The nature of the 5, 5'- substituents barbituric acid in itself is a strong acid, having a p*Ka*of 4.01 in water.3a It is partially soluble in polar solvents, such as methanol and water, and in these solvents retains its acidic properties, as well as be converted into the corresponding salt when treated with a base.3b Generally speaking, barbiturate derivatives having at least one unsubstituted NH hydrogen retain their acidic properties, but the relative acidity of barbituric acid derivatives depends not only on the N-substitution, but the *C*-5 substitution as well (Figure 1.10) [37].



Barbituric acid Pka= 4.04 5, 5'- disubstituted barbituric acid pka-6,5,8 3,5,5'-trisubstituted barbituric acid pka->8

Figure 1.10: Acidic properties of barbituric acid

The barbituric acid ring itself contains one sp3 hybridized carbon atom, and is treated as an achiral ring system unless the 5,5'-substituents differ from one another and one of the NH moieties is substituted 3.

1.3.4 Pharmacological effects of barbituric acid

Historical literature accounts describe barbituric acids derivatives as exhibiting a primary mode of action on the central nervous system. There have been more recent literature documentation that barbituric acid derivatives can exhibit biological activities in other areas, such as anti-bacterial, anti-fungal, possess anti-cancer activity, anti-osteoporosis activity cytotoxic activity [35].

1.4 Antibacterial Activity and Antifungal activity

Antibacterial are used to treat bacterial infections and Antifungals are used to kill or prevent further growth of fungi. The health problem demands to search and synthesize a new class of antimicrobial compounds which are effective against pathogenic microorganisms and develop resistance to the antibiotics used in the current regime. The increasing resistance of human pathogens to current antimicrobial agents is a serious medical problem. During the 20th century, vaccines for bacterial toxins and many other

common acute viral infections were developed and made widely available. The incidence of fungal infections has increased significantly in the past two the pyrimidine derivatives possess a wide variety of potentially biological properties and are well known to work as herbicides and pesticides [38].

Therefore pyrimidine derivatives of barbituric acids are becoming a very important subject of medical research. Barbituric acid have been widely investigated and known for their antibacterial, antifungal, activities.

1.5 Objectives of the Research Work

In medicinal chemistry pyrimidine derivatives have been very well known for their therapeutic application. Over the years, the pyrimidine system turned out to be an important pharmacophore donated with drug like properties and a wide range of pharmacological activities depending on the skeleton.

The objective of the present work is to isolate all possible synthesized products formed in these reactions. The specific aims of these studies are:

- to synthesis the pyrimidine derivatives;
- to identify all possible products of this reaction;
- to evaluate their structure by spectroscopic method;
- to study its antimicrobial activity and Antifungal activity;
- to optimize the yield of the products by varying the parameters such as catalysts, solvents and temperatures.

CHAPTER II

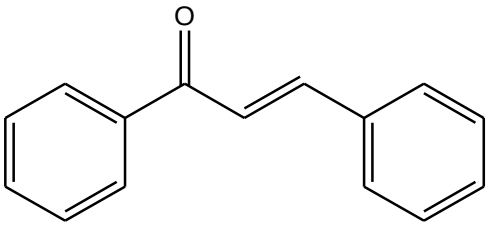
LITERATURE REVIEW

CHAPTER II

Literature Review

2.1 Literature survey on the Synthesis of Pyrimidine Derivatives and Investigation on their biological Activity.

Patil *et al.* reported on a versatile moluclefor the synthesis of chalcones, (01) and its chemical modifications to flavonoids, flavanone, pyrazoles, oxazoles, pyrimidines. This



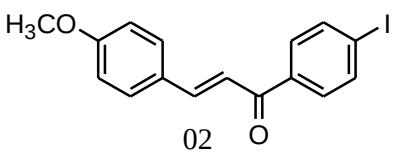
01

article also highlights antioxidant potential of chalcone, mechanism of antioxidant activity of chalcones and structure activity relationship of chalcone derivatives for antioxidant ability and different methods to evaluate antioxidant activity of chalcone, antiinflammatory, cytotoxic and antihyperglycemic activity of chalcones [39].

Jyothi *et al.* studies on the antibacterial activity of synthesized chalcones and their pyrimidine derivatives, and their activity conducted against three gram-positive bacteria viz., Bacillus *pumilis, Bacillus subtilisandStaphylococcus aureus*and two gram-negative bacteria viz., *Escherichia coli, Proteus vulgaris*byusing cup plate method. All the compounds which were screened for antibacterial activity, also screened for their antifungal activity. The fungi employed for screening were Aspergillusniger, Rhizopusnoryzaeand *Candida albicans* [40].

Choudhary *et al.* have also studied on the chalcone which have been synthesized by condensingbenzaldehyde derivatives with acetophenone derivatives in dilute ethanolic sodium hydroxide solution sharp bands in the range between 1630-1660 cm^{-1} indicated the

presence of C=O group. The results revealed that majorityof the synthesizedcompounds showed varying degrees ofinhibition against Gram positive bacteria [38] The 02 showed excellent activity against *S. aureus*at both concentration i.e. 500 μg/ml and1000 μg/ml.



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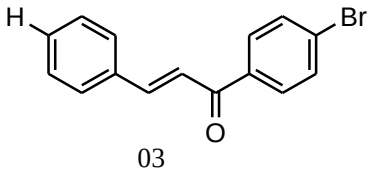

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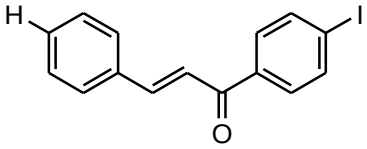

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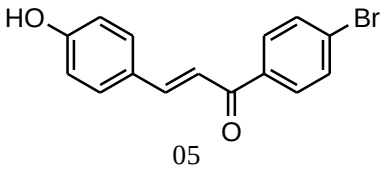

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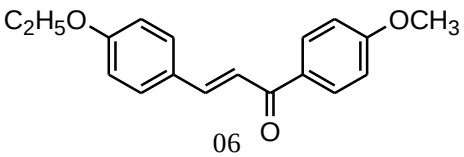

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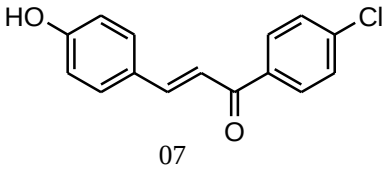

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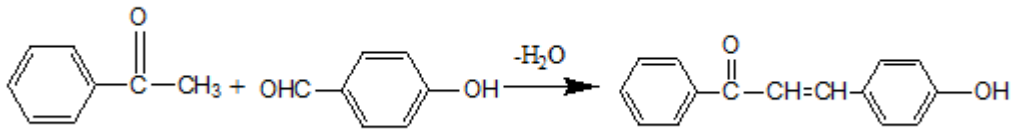
The compounds 02, 03, 04, 05, 06 and07 have shown good to moderate activity against *S aureus* at both concentration i.e. 500μg/ml and 1000 μg/ml. Three of the chalcones with antistaphylococcal activity (04, 05 and 06) gave no inhibitory zones probably due to their low diffusion potential intoagarmedia. Finally, no activity was observed for compounds against *P. aeruginosa*, a gram negative organism [41].

Rahman *et al.* studied on potential pharmacological activities of chalcone. From literature survey on it was found that almost all chalcone precursors of open chain flavonoids and isoflavonoids present in edible plants. It can be said that chalcones and their derivatives display a wide range of pharmacologicalactivities, such as antimalarial, anticancer, antiprotozoal (antileishmanial and antitrypanosomal), antiinflammatory, antibacterial, antifilarial, antifungal, antimicrobial, larvicidal, anticonvulsant and antioxidant activities. They also show inhibition of the enzymes, especially mammalian alpha-amylase, cyclooxygenase (COX) and monoamine oxidase (MAO) and antimitotic activity too. Because of this, chalcones and their derivatives have attracted increasing attention of the scientists for the search of new potent pharmacological activity in it [42].

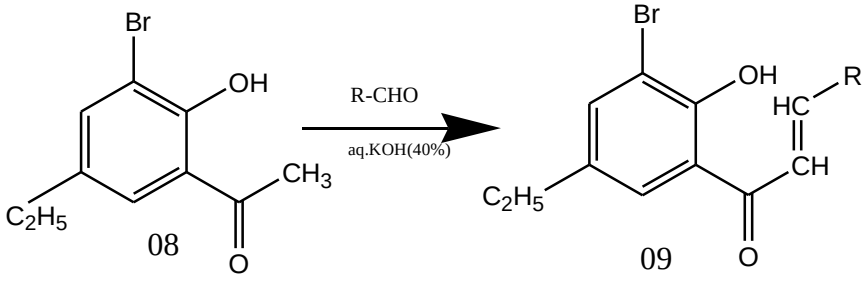
The synthesis and evaluation of chalcone derivatives for its alphaamylase inhibitory activity were reported by Attarde *et al.* They show that of the synthesized compound 3-(4-hydroxyphenyl)-1-phenylprop- 2-en-1-one has an alpha amylase inhibitory activity [43].



Acetophenon 4-Hydroxy benzaldehyde 3-(4-hydroxy-phenyl)-1 phenyl-propenone

Scheme: 2.1

Naik *et al.* concluded some synthesized chalcone derivative from 2-hydroxy-3-bromo-5-ethyl acetophenone (08).



Scheme: 2.2

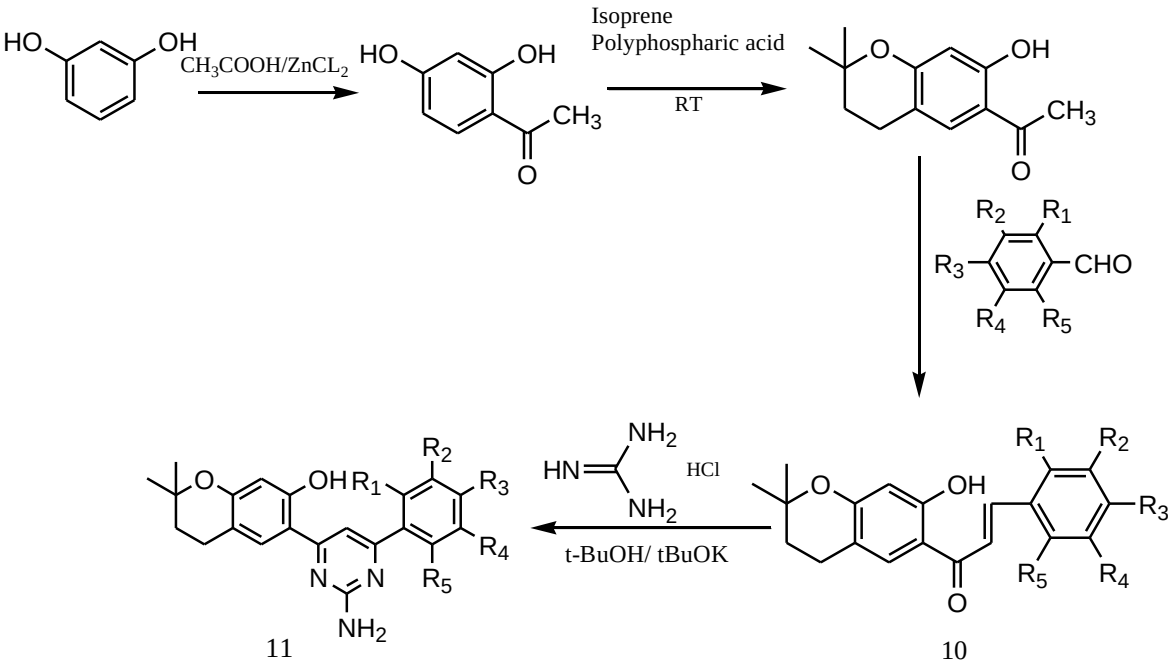
The chalcones are associated with different biological activities like insecticidal, anticancer, anti-inflammatory, bactericidal, fungicidal, antiviral, antitumor, antimalarialand antiulcer. Literature shows that lieochalcone and oxygenated chalcone has strong antileishmanial activity. It is reported that chalcones exhibited potent activity against human malarial parasite [44]**.**

Nadia *et al.* was reported that some substituted 4, 6-diaryl-3-cayno-2-thioxopyridine derivatives were synthesized from appropriate substituted 1, 3-diphenyl prop-2-en-1-one (Chalcone) reaction with cyanothio acetamide. The antibacterial and antifungal activity data of the prepared compounds showed good antimicrobial activity [45].

Raj *et al.* reported that on various Novel heterocyclic derivatives. All synthesized compounds as well as the reactions that carried out were characterized and monitored by TLC, melting point, nitrogen estimation, IR and 1H NMR and they all gave satisfactory results evaluated for their antibacterial activity against various types of bacteria. The compounds have shown significant antibacterial activity in comparison against Norfloxacin and Ampicillin at 100mcg/mL. So all the synthesized compounds showed activity [46].

Kandeel *et al.* has been reported for their antitumor activity by *in-vitro* disease-oriented human cells screening panel assay revealed that all the synthesized compounds exhibit moderate cytotoxic activity especially against renal cancerUO-31cell line, particularly some compound which shows inhibition 57.11%. The pyrazoline derivatives are more potent than other cyclized derivatives as anticancer agents. While the isoxaline derivative show only mild antitumor against renal cancer UO-31 (34.93%).Mean while, the pyrimidine derivatives show moderate antitumor activity [47].

K. Suneel *et al.* reported that the chalcones (10) which were synthesized have now have been taken for the preparation of corresponding new Pyrimidine derivatives (11). The condensation above chalcone with guanidine hydrochloride in alkalinemedium viz., in potassium tertiary butoxide in presence of tert-butanol at reflux temperatures and resulted the formation of corresponding Pyrimidine derivatives. The synthesized chalcone derivatives were undergone physicochemical characterization and the obtained results.



Scheme: 2.3

The yields of the synthesized compounds were found to be significant. The structure of the synthesized compounds was confirmed by IR, Mass and elemental analysis. Elemental analysis showed that the percentage of the nitrogen, hydrogen and carbon was found experimentally is equivalent to the calculated values in all compounds [48].

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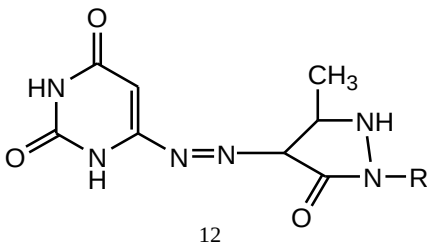
Chaturvedi *et al.* found that efficient and convenient procedure for the synthesis of benzimidazole substituted pyrimidine ligands and their Bi (III) metal complexes. Moreover in microwave technique theiractions can be carried out under less solvent conditions which play a strategic role as the solvents are often very toxic, expensive and difficult to use. Hence the use of microwave technique for the synthesis of organic and inorganic compounds will bethe part of "green chemistry" The antimicrobial activity of free pyrimidine ligands and their Bi (III) metal complexes in 1:2 molar ratios were examined. These activities were compared with known antibiotics such as Streptomycin and Fluconazole. It is observed that metal complexes showed better activity than the activity of free ligands but lower activity than the activity of standard drugs. Therefore it may be concluded that the complexation of metal moiety withbioactive pyrimidine ligand increases the biological activity of the complexes [49].

Nehad *et al.* also reported the reactions and antimicrobial activity of some substituted 4, 6-diphenyl pyridine 2-thione derivatives. The synthesized compounds exhibit significant antibacterial activity against pathogenic bacteria. The characterization of the resulting products was confirmed by FTIR, ^{1}HNMR, MS and elemental analyses. The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli, Pseudomonas aurignosa, Salmonella typhimurium, and Bacillus subtilis and Staphylococcus aureus* using the disc diffusion method [50].

Arikkatt *et al.* concluded that pyrimidines are synthetically versatile substrates and hence can be used for the synthesis of a large variety of heterocyclic compounds.This heterocyclic moiety has great biological and medicinal significance. Various synthetic aspects indicate that pyrimidine derivatives are easy to synthesize which can produce a wide variety of activity [22].

Nofal *et al.* was reported that all the tested compounds showed significant analgesic effect due to the presence of heterocyclic pentaatomic nucleus (pyrazole, pyrazolone, pyrazolidindione) at position 5 of the pyrimidine moiety. Compounds 12, and 15 showed longer withdrawal latency compared to tramadol due to thepresence of phenyl group at position 1 of pyrazolone or pyrazole nucleus. Compound 13 showed the most prompt andstrongest anti-inflammatory effect due to the presenceof 3-chlorophenyl group at position 1 of pyrazolone. On the other hand, compound 14 showed the most sustainable anti-inflammatory effect which was comparable to indo methacin due to the presence of carboxamide group at position 4 of pyrazolone [51] Shown in Fig.



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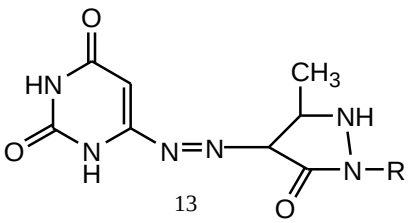

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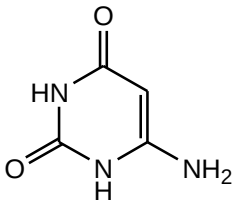

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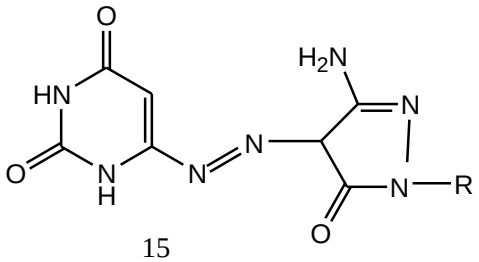

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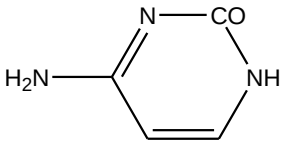
Dansena *et al.* have been reported that Research investigates that pyrimidine derivatives show various important pharmacological activities. Not much of the research is done in screening pyrimidine derivatives for in-vitro and in-vivo pharmacological activities.



Cytosine

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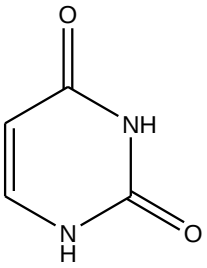

Uracil

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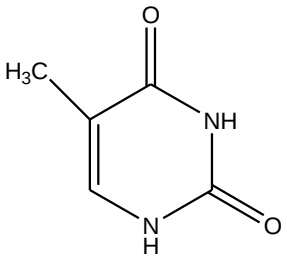


Thymine

18

Thus, a tremendous scope for research is present in this direction for investigating pyrimidine derivatives as lead molecules [28].

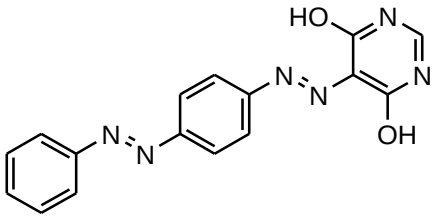
Kashyap *et al.* was reported also that focus on the various synthesis and pharmacological activity of the thiazolopyrimidine. After literature review it is concluded that 5-(4-chlorophenyl)-2-(4-fluorobenzylidene)-7-methyl-3-oxo-2,3-dihydro-5H-thiazolo pyrimidine-6-carboxylic acid (4-fluoro phenyl) amide and (2Z)-2-((E)-2-((1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)methylene)hydrazono)-2,3,5,6-tetrahydro-3-phenyl-5-thioxothiazolo pyrimidin-7(4H)-one derivatives have great potential of anti-inflammatory activity, it revealed that the substitution of substituted benzene and pyrazole moiety enhance the anti-inflammatory activity of the compounds . The series of 4-(2-Aminothiazol-4-yl)-3-methyl-5-oxo-1-phenyl-2-pyrazoline and various substituted thiazoloprimidine derivatives have efficient antimicrobial activity against various fungal and bacteria.It observed that cyclic structure with minimum substitution with electron withdrawing groups like C_2H_5, -OH and fluoro were showed good potential activity and electron donating groups like $–NH_2$, $-CH_3$

showed less active compounds. Substitution of electron withdrawing group also showed better antiviral and anticancer activity [52].

Verma *et al.* concluded that the various derivatives of pyrimidine showed various important pharmacological activities like antibacterial compound 2-(6' fluoro benzothiazol-2'- ylamino)-4,6- (disubstitutedthiouriedo)-1,3- pyrimidine have shown best activity against different strains of bacteria viz., *B. subtilis, E. coli, P. aeruginosa* and *S .aures*using agar diffusion techniques. Various other activities are also been studied like anti-inflammatory, analgesic, hypnotic, antihistaminic, mollucicidal, antinociceptive, steroidal, cytotoxic and anticancer agents. Thus by all studying all the derivatives showing variety of activities can say that pyrimidine ring have been explored in past years and is still be used for future development of new drugs against many more pathological conditions [53].

According to the recent literature survey, it may be observed that novel pyrimidine derivatives are found to have potent antimicrobial activities and this information has been summarized in this section as given below:

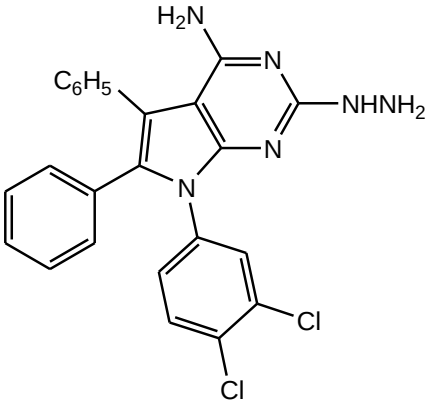
Yazdanbakhsh *et al.* suggested that the synthesis of 4, 6-dihydroxypyrimidines (compound 19) and evaluated for their antibacterial activity against *Salmonella typhimurium, Micrococcus luteus, Bacillus subtilis*and *Pseudomonas aeruginosa* atconcentration of 125µg/ml by using tetracycline and erythromycin as standard drugs. One compound showed good antibacterial activity against *B. subtilis* and *P. aeruginosa* when compared with standard drugs [54].



19

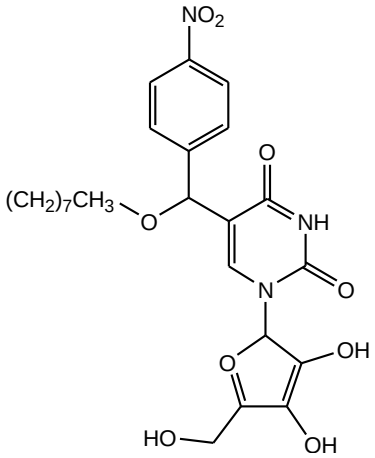
A series of 8-Aryl-pyrrolo, thiazolopyrimidine derivatives (20) prepared by Fatahalaa *et al.* all newly synthesized compounds were examined for their antibacterial activity against Staphylococcus pyrogens and antifungal activity against *Candida albicans* and *Aspergillus flavus* according to cup plate method at a concentration of 0.035mg/ml by using

Chloramphenicol and Flucanazole as standards for antibacterial and antifungal activities respectively. Some compounds showed promising antimicrobial activitieswhen compared with their respective standard drugs [55].



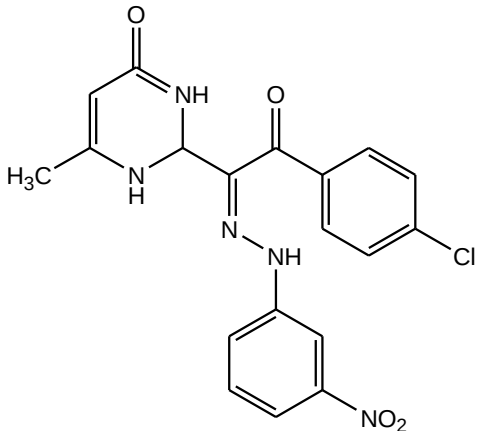
20

Again a series of uridine analogues (21) was synthesized by Kharb *et al.* and tested for their antibacterial activity against *Pseudomonas aerugenosa* and *Staphylococcus aureus* and antifungal activity against *Aspergillus niger* and *Candidaa lbicans*. Chloramphenicol and Flucanazole were used as standards for comparison of antibacterial and antifungal activities respectively. Some compounds demonstrated significant antibacterial activity against *P. aerugenosa* and *S. aureus* whereas other compounds exhibited appreciable antifungal activity against *A. niger* and *C. albicans* when compared with their respective standard drugs [56].



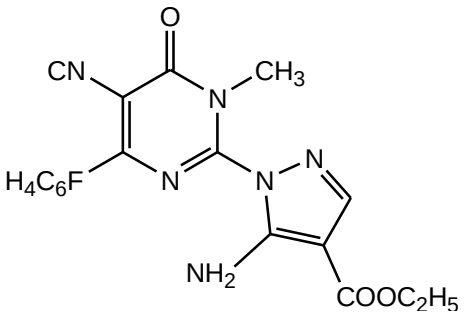
21

Edrees *et al.* prepared a series of 2-[N-aryl-2-oxo-2-(4-chlorophenyl) ethanehydrazonoyl]-6-methyl-4(3H) pyrimidinones derivatives (22). All newly synthesized compounds were screened fortheir potent antibacterial activity against *Pseudomonas aerugenosa* and *Staphylococcus aureus* and antifungal activity against *Aspergillus niger* and Candida albicans. Chloramphenicol and Flucanazole were used as standards. Some compounds showed promising antibacterial activity against *P. aerugenosa* and *S. aureus* whereas other compounds exhibited significant antifungal activity against *A. niger* and *C. albicans.* One compound showed good antimicrobial activity at minimum inhibitory concentration of 15.10µg/mL against the bacterial strains [57].



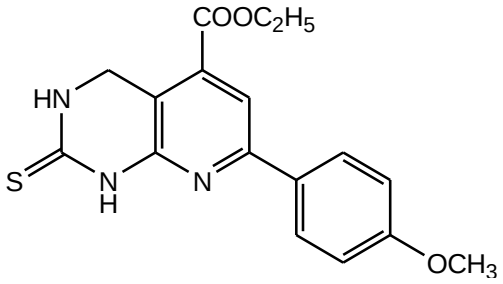
22

Ramesh *et al.* synthesized novel dihydropyrimidines derivatives (23)and their in-vitro evaluation for antibacterial activity against gram positive bacteria *(Enterococcus fecalis* ATCC-29212, *Bacillus subtilis)*and gram negative bacteria (*Escherichia coli* ATCC-25923, *Pseudomonas aeruginosa*ATCC-27853) and antifungal activity against(*Candidaalbicans* NLTM-3431, *Aspergillusniger*) were compared with their respective standard drugs Amoxycillin and Griseofulvin. Some compounds showed significantantimicrobial activity when compared with standarddrug. One of these novel derivatives showed potent in vitro antimicrobial activity at concentration of 15.10µg/mL as compared to the standard drug streptomycin and also reported antibacterial activity [58].



23

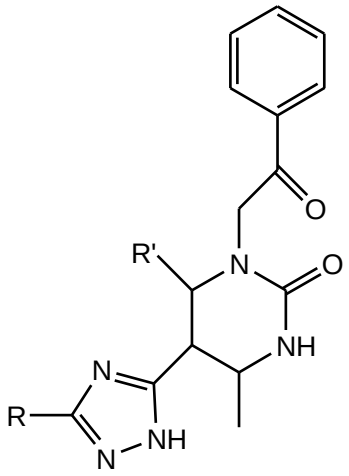
Dudhe *et al.* synthesized pyridopyrimidines derivatives (24). The in vitroantimicrobial activity of some of the newly synthesized compounds was examined. All the tested compounds proved to be active as antibacterial and antifungal agents. One compoundshowed potent activity against various bacteria tested in comparison of standard drugs chloremphenicol and nystatin [59].



24

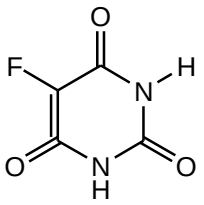
Joshi *et al.,* has concluded that a series of novel pyrimidine derivatives were synthesized from chalcones and evaluated for their pharmacological a new class of different pyrimidine synthesized and evaluated for anti-inflammatory and analgesic activities. Among the tested fluoro pyrimidine moiety showed better anti-inflammatory activity while chloro pyrimidine andfluoro pyrimidine moiety showed better analgesic activity. It can be concluded that pyrimidine class of compounds synthesized from activities chalcones certainly holds great promise towards good active leads in medicinal chemistry. A further study to acquire more information concerning pharmacological activity is in progress [60].

Singh *et al.* have synthesized various derivatives of pyrimidines. The fungicidal activities of the compound were evaluated against *P. infestans* and *C. falcatum* by the usual agar plate method (structure 25)



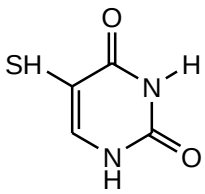
25

The pyrimidine moiety with some substitution gave promising antitumor activity as there are large numbers of pyrimidine based antimetabolites. Early metabolite prepared was 5-fluorouracil (26), a pyrimidine derivative followed by 5-thiouracil (27) which also exhibits some useful antineoplastic activities [61].



5-Flurouracil

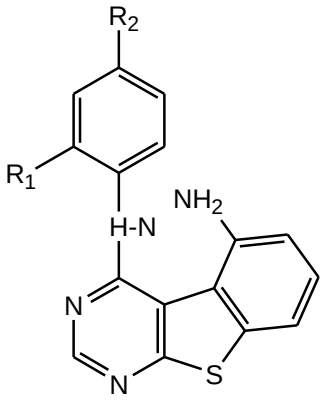
26



5-Thiouracil

27

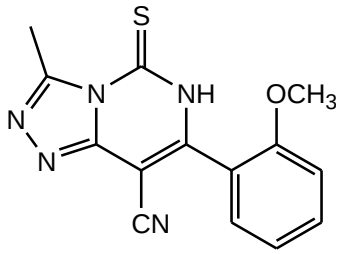
Pedeboscq *et al,* [62] has synthesized 4-(2-Methylanilino)benzo[b]thieno [2,3d]pyrimidine and 4-(2-Methoxyanilino)benzo[b]thieno [2,3-d]pyrimidine(2)(28), which showed a similar cytotoxicity to the standard anti- EGFR geftinib suggesting a blockade of the EGFR pathway by binding to the tyrosine kinase receptor.



R_1=CH3, R_2=H for(1)
R_1=$OCH_{3,}$ R_2=H for (2)

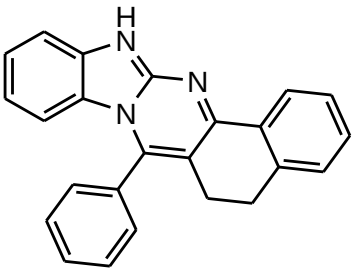
28

Fathalla *et al*. has synthesized a series of some new pyrimidine derivatives like 7-(2-methoxyphenyl)-3-methyl-5-thioxo-5,6-dihydro[1,2,4]-triazolo[4,3-c]pyrimidine-8-carbo-nitrile via reaction of ethyl cyanoacetate with thiourea and the appropriate aldehydes namely 2-methyl-benzaldehyde and 2-methoxy-benzaldehydefollowed reaction with different reagents [63]. All structures were than screened for bacterial activity and anticancer activity (29).



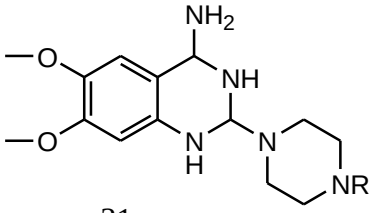
29

Lee *et al*. has synthesized some novel pyrimidines derivative having thiazolidinedione. These compounds were evaluated for their glucose and lipid lowering activity using pioglitazone and rosiglitazone as reference compound (30).



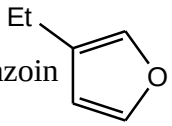
30

Many pyrimidine ring containing drugs have exhibited antihypertensive activity. A quinozoline derivative, prazone, is a selective α1- adrenergic antagonist.Its related analogues bunazosin, trimazosin and terazosin are potent antihypertensive agents [64] Fig below



31

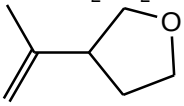
R= for prazoin



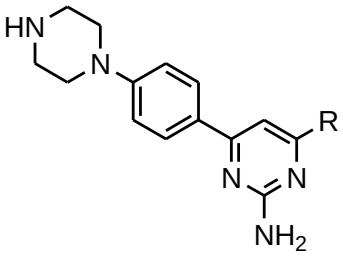
R= forBunazosin-$CH_2OOCH_2OOH(CH_3)_2$

R= for Trimazosin-$COCH_2CH_2CH_3$

R= for Terazosin

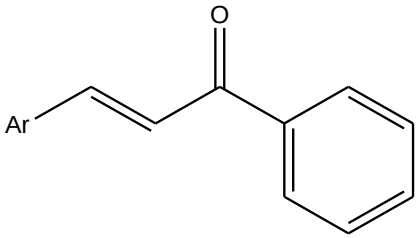


Rahaman (2011) *et al*. has synthesized nobelpyrimidines by the condensation of chalcones of 4′-piperazineacetophenone with guanidine HCl Fig. It shows significant antihistaminic activity when compared to the reference antihistaminic drug piramine [65].



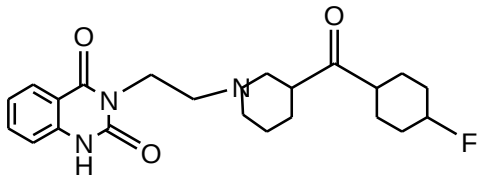
32

Ebraheem *et al.* evaluted An efficient synthesis of chalcones (33) was achived by condensation of substituted benzaldehyde with substituted acetophenone in ethanolic sodium hydroxide (50%).The chalcones were reacted with urea and thiourea to give the pyrimidinones and pyrimidinethiones respectively. All the prepared compounds were confirmed by the available physical and spectral methods.

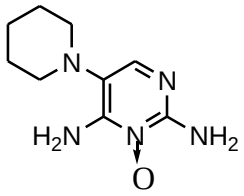


33

Ganzevoort *et al.* minoxidil has a similar effect and is an antagonist of both a1-adrenergic and serotonin-S2 reporters. A triaminopyrimidine derivative, minxidil, whose mechanism of action and therapeutic action are similar to prazosin, has been introduced in therapy for its side effects, in the treatment of alopecia, male baldness etc [67] structure (34,35).



34



35

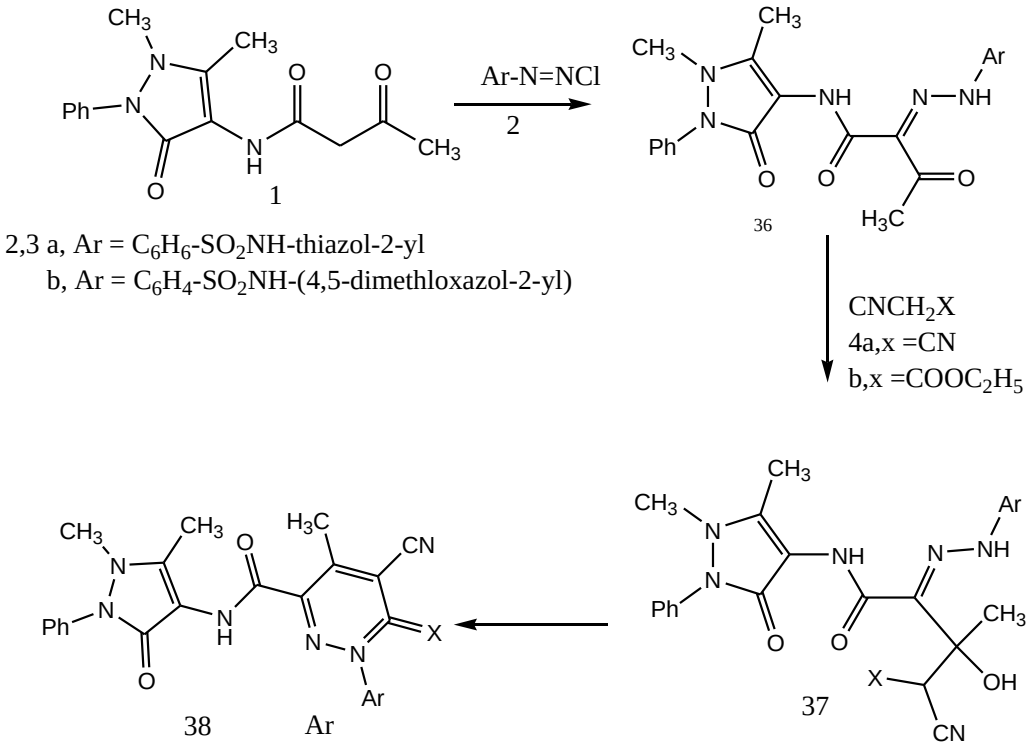
Rahaman *et al.* reported (2009) thatsynthesized pyrimidine derivatives showed asignificant broad spectrum antibacterial activity at concentration levels 0.05% (50µg/ml) and 0.1 %(100µg/ml) respectively. When an increase in concentration of all the synthesized pyrimidine derivatives showed an increase in zone of inhibition.These measured zone of inhibitions suggested that the synthesized compounds exhibited a significant antibacterial activity. Among all the Pyrimidine derivatives particularly halogen substituted derivatives showed more activity than others [68].

Gouhar *et al.* was reported that synthesis of novel derivatives of pyrazole nucleus attached to or fused with various heterocyclic ring systems such as imidazole, tetrazole and (thiopyrimidine rings. Some of the newly synthesized compounds were examined as anticancer agentsagainst three different carcinoma cell lines (MCF-7, HCTH-6, HePG-2). The derivatives 3, 5-diaminopyrazolyl 13 and 4, 6-diaminopyrimidinyl 15b appeared to be of potential cytotoxic activity [69].

Chaturvedi *et al.* was reported that on investigation is a comparative study between traditional (conventional) and microwave technique of metal complexes of Bi (III) with pyrimidine derivatives as a ligand and also determining their antimicrobial activity against selected organisms. The metal complexes of Bi (III) appear to acquire the coordination number five and have distorted octahedral geometry. The antimicrobial activity of free pyrimidine ligands and their metal complexes were determined. These activities were compared with the activity of known antibiotics such as Streptomycin and Fluconazole [70].

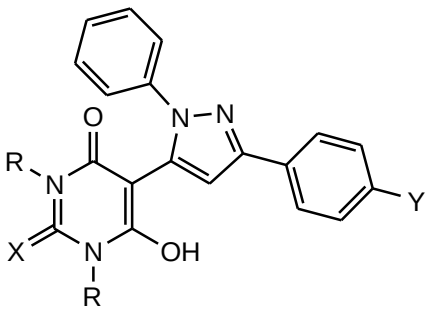
Saini *et al.* reported the same work on pharmacological significances of pyrimidine derivatives. Literature reveals no of methods for the synthesis of pyrimidine derivatives and also their diverse reactions offer enormous scope in the field of pharmaceutical chemistry. Pyrimidine possess wide spectrum of biological activities like anti-inflammatory, antifungal, antibacterial, antitubercular, antiviral, antimalarial, anticancer, anti-HIV activity. The present review attempts to give brief information about the synthesis and various biological activities of pyimidines and their derivatives [71].

Ismail, M *et al.* has been reported the derivatives of new hydrazo, pyridazine, thienopyridazine, pyrazolopyridine, pyridopyrazolopyrimidine, and pyridopyrazolotriazine that are expected to have biological activities have been synthesized and their structures confirmed by their spectral data, elemental analyses, and with some chemical reactions [72]. Reaction shown below,



Scheme: 2.4

Kumar *et al.* reported that pyrimidine pyrazoleheterocyclies can be of interest as 39g showed significant antibacterial activity. The compounds 39e and 39h also showed moderate antibacterial activity. 39j showed moderate antifungal activity.

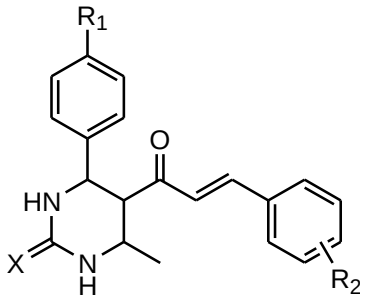


39

Compounds	R	X	Y
39c	$-CH_3$	O	Br
39e	$-C_6H_5$	S	CH_3
39g	$-C_6H_5$	S	Br
39h	$-C_6H_5$	S	Br
39j	$-C_6H_5$	S	OCH_3

Out of all heterocycles, 39c possesses both antifungal and antibacterial activity. Our studies showed that these novel heterocycles can supplement the existing antifungal therapy. Monotherapy can be replaced by combination therapy. Therefore 39c, 39g, and 39j might be of great interest for the development of novel antimicrobial molecule [73].

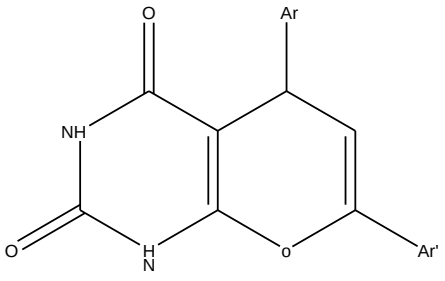
Shaikh *et al.,* concluded that synthesized new 3, 4-dihydropyrimidine derivatives via novel chalcone series. We studied the biological importance of synthesized compound 40 by screening their antimicrobial activity against four fungal and bacterial microorganisms.



40

All the synthesized compound 40a-rshowed good antibacterial activity against all the test microorganism while compounds 40c, 40d, 40k, 40p and 40q exhibited most potent antifungal showing highest inhibition zone against *Aspergillus fumigatu* and *Candida parapsilosiss* [74].

Khatun *et al.,* studied the attachment of barbituric acid with arylideneacetophenone under microwave irradiation (MWI) and conventional heating. In conventional heating, the yield of the compounds (41) were very poor (77-81%), but in MWI methods the yields were observed 96-98% which was comparatively too high.



	Ar	Ar'
41a	C_6H_5	C_6H_4OH
41b	C_6H_4Cl	C_6H_4Cl
41c	C_6H_4Cl	C_6H_4OH
41d	$C_6H_4CH_3O$	C_6H_5
41e	$C_6H_4CH_3O$	C_6H_4Cl

41

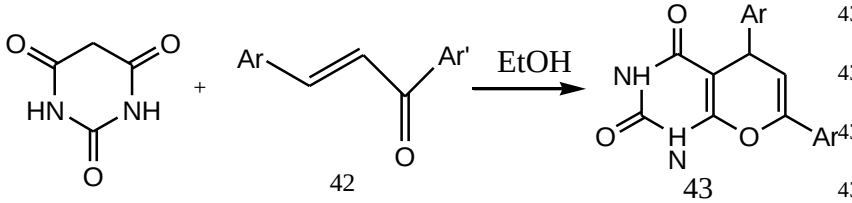
The structures of the compounds were characterized by FT-IR, 1H-NMR spectral data. The antimicrobial and cytotoxic activities of the synthesized compounds were also investigated. All the tested bacteria revealed the zone of inhibition were 6-13 mm where sample concentration was 100μg/disc. The presence of a reactive and unsaturated ketone

function in synthesized compounds was found to be responsible for their potential antimicrobial and cytotoxic activity [75].

Saad *et al.* have also studied that microwave assisted synthesis of some New fused 1,2,4-Triazines Bearing thiophene moieties with expected pharmacological activity by treatment of with bifunctional oxygen and halogen compounds, CS/KOH and malononitrile via heterocyclization reactions, in addition to some uncondensed triazines. This work revealed that product compounds may be active cytotoxic agents against different cancer cell lines. This cytotoxic effect was found to be mainly due to apoptosis, which indicated that those compounds may be promising candidate anti-cancer agents, subject to further study. From the chemistry point of view, cytotoxic effect may be due to the presence of free thiol or thioether groups in these compounds [76].

There has been a report on chemical and pharmacological potential of various substituted thiazine derivatives by Asif *et al.* thiazine compounds possess variety of pharmacological activities like anti-microbial, anti-mycobacterial, antifungal, antiviral, antitumor, antipsychotic, anti-inflammatory etc. The significance of thiazine derivatives has potential pharmacological moiety and future of these derivatives in the field of drug research. Some of the pharmacological activities are briefly summarized. This article summarizes various chemical reactions like condensation, cyclo-addition, ring transformations etc [77].

Sattar *et al.* reported that (2015) the rapid and efficient procedure for the attachment of barbituric acid with arylideneacetophenone under microwave irradiation (MWI) and conventional heating. In conventional heating, the yield of the compounds (43) were very poor (75-81%), but in MW methods the yields were observed 96.48-98% which was comparatively too high.

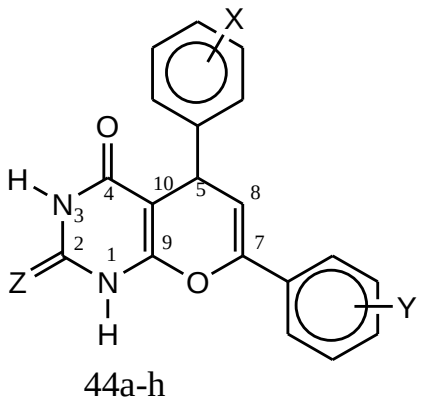


	Ar	Ar'
43a	C_6H_5	C_6H_5
43b	C_6H_4OH	C_6H_5
43c	C_6H_5	C_6H_4Cl
43d	C_6H_4OH	C_6H_4Cl
43e	C_6H_4Cl	C_6H_5

Scheme: 2.5

The structures of the compounds were characterized by FT-IR, ^{1}H-NMR spectral data. The antimicrobial and cytotoxic activities of the synthesized compounds were also investigated. The presence of a reactive and unsaturated ketone function in synthesized compounds was found to be responsible for their potential showed antimicrobial and cytotoxic activity [78].

Akhter *et al.* also reported on one step cyclo condensation of (thio) barbituric acid with chalcones in glacial acetic acid and phosphorous pentoxide and concluded that 5, 7-diaryl-1,5-dihydro (or 1, 2, 3, 5-tetrahydro)- pyrano[2, 3-d] pyrimidin-2, 4-diones (or 2-thioxo-4-ones) 44 (a-h) have been synthesized in one-step by cyclocondensation of barbituric acid or thiobarbituric acid with arylideneacetophenones, in glacial acetic acid in the presence of phosphorous pentoxide.



44a-h

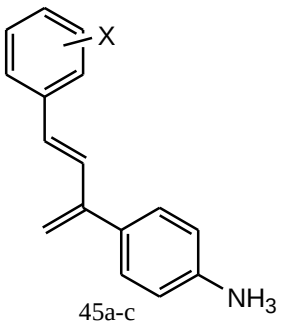
Substituents	X	Y	Z
44a	4-Cl	4-Cl	O
44b	4-Cl	4-Cl	S
44c	4-Cl	4-NH_2	O
44d	4-Cl	4-NH_2	S
44e	2-Cl	4-Cl	O
44f	2-Cl	4-Cl	S
44g	2-Cl	4-OCH_3	O
44h	2-Cl	4-OCH_3	S

The structures of the compounds 44(a-h) have been determined by UV, IR, ^{1}H NMR, ^{13}C NMR, mass spectral data and elemental analyses. The compounds 3a-h does not seem to be available in the literature [79].

Ikotun and co-workers stated that cobalt (II), copper (II), iron (III), manganese (II) and nickel (II) complexes of barbituric acid were synthesized and characterized by infrared and ultraviolet spectroscopy. The ligand and the complexes have been screened for in vitro antibacterial activity against a range of gram-positive and gramnegative bacteria *(Bacillus authracis, Bacillus cereus, Bacillus stearothemophilus, Bacilis subtilis, Escherichia coli,*

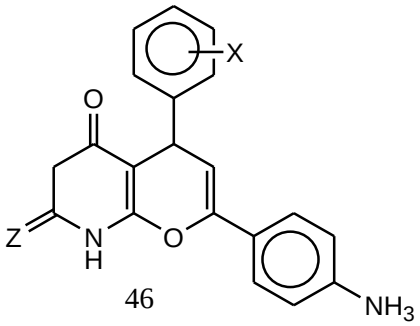
Klebsiella pneumonia, Pseudomonas aeruginosa, Pseudomonas fluorescens, Staphylococcus aureus, Streptococcus faecalis). Cobalt (II) complex displayed a better antibacterial activity than the ligand, showing a broad-spectrum activity against all the six gram-positive and four gram-negative bacterial strains, while the other complexes showed only selective activity against theorganisms in a manner similar to the starting ligand [80].

Nasrin *et al.* reported that on the microwave assisted synthesis of pyrano [2, 3-d] pyrimidinone derivativesand stated that the starting materials 45(a-c), a series of the fused compounds of barbituric and thiobarbituric acid. 7-(4-aminophenyl)-5-aryl-5H-pyrano [2, 3-d] pyrimidinone 56(a-f)were synthesized in aqueous ethanol.



45a-c

45a, X=H
45b,
45c X-2Cl



46

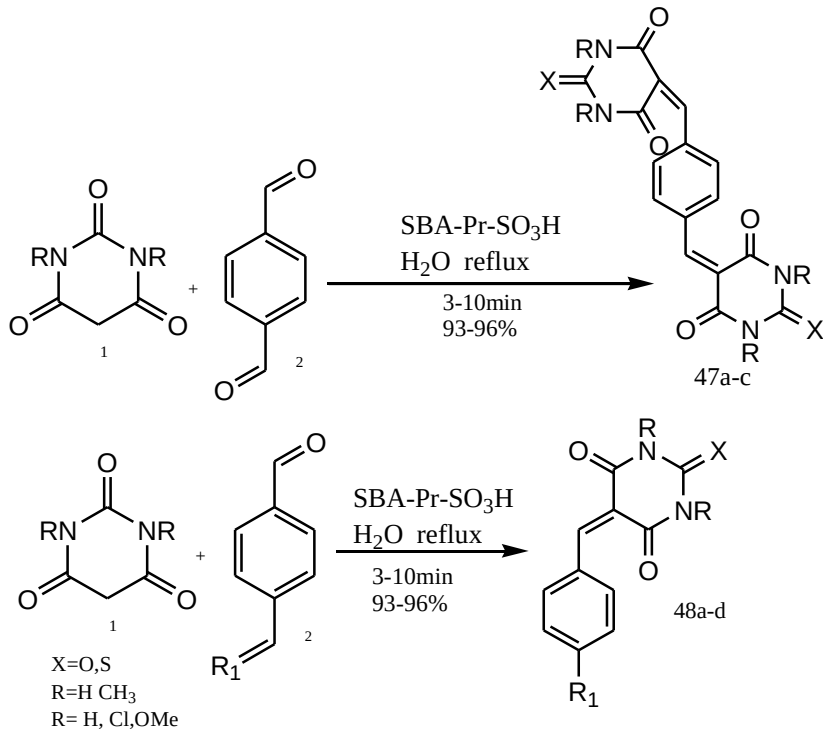
46a, X=H Z=O
46b, X=H Z=S
46c, 4-OCH_3 Z=O
46d, 4-OCH_3 Z=S
46e, X=2-Cl Z=S
46f X=2-Cl Z=S

55

All reactions were carried out in a microwave oven with special fabricated glassware and optimum reaction conditions were determined. These synthesis apart from reducing the use of organic solvents from work up step, also gave improved yield as compared to the conventional heating with reaction time reduced from hours to minutes. Low amount of chemicals were used making the method of synthesis environmental friendly. In other words this modest work was a part of 'green chemistry' too [81].

There has been reported on the knoevenagel condensation using sulfonic acidfunctionalizednanoporous silica (SBA-Pr-SO_3H) by Ziarani *et al.* that Knoevenagel condensation between barbituric acid and aldehyde was in the presence of sulfonic acid

functionalized nanoporous silica (SBA-Pr-SO_3) and resulted in the formation of arylidene and bis-arylidene barbiturates [82].



Scheme: 2.6

Excellent yields and short reaction times are related to the high efficiency of SBA-Pr-SO_3, that the reactions take place easily in its nano-pores. SBA-Pr-SO_3 as an efficient heterogeneous nanoporous solid acid catalyst which was prepared by silica functionalization with (3-mercaptopropyl) ntrimethoxysilane followed by oxidation with H_2O_2, can be easily removed from the reaction mixture by simple filtration, and also recovered and reused without noticeable loss of reactivity.

Mst. Khodeza Khatun *et al.* was reported that comparison of microwave irradiation and conventional synthesis of 2- thiobarbituric acid derivatives and in vitro Evaluation of antimicrobial and cytotoxic activity. The microwave-assisted syntheses offer reduction in reaction time, excellent yields without any undesirable side products, cleaner reaction and operation simplicity. Microwave synthesis method also reduces the reaction time from hours to minutes with improved yield as compared to the conventional heating. The use of low amount of chemicals proves this synthesis method as environmental friendly. In other

words, as a recent work of green chemistry, it is very useful for performing the synthesis of drug, intermediates and chemicals [16].

Hosseini and coworkers also reported that one-pot new barbituric acid derivatives derived from the reaction barbituric acids with BrCN and ketones .They obtained Structure elucidation was carried out by x-ray crystallographic, ^{1}HNMR,^{13}C NMR, two dimensional NMR, FT-IR spectra, mass spectrometryand elemental analysis. The mechanism of product formation is discussed. The reaction of DMBA with cyanogen bromide in the presence of triethylamine also afforded trimeric form of barbiturate of uracil derivatives in good yield. The reaction of selected acyclic βdicarbonyl compounds with cyanogen bromide in the presence of triethylamine in acetone and/or diethyl ether has also been investigated under the same condition. Diethyl malonate and ethylcyanoacetate brominated and also ethyl acetocetate both bro-minated and cyanated on active methylene via cyanogen bromi [83].

CHAPTER III

EXPERIMENTAL

CHAPTER III

Experimental

3.1 General

All chemicals and solvent will be purchase from BDH or Merck chemical company. Melting point was measuredfrom our laboratory , FTIR spectra and NMR will carried out at CARS in Jahangirnagor University.

3.2 Solvent

Drying of alcohol: One liter commercial grade ethanol was taken in taken in two 500 ml conical flasks containing freshly ignited calcium oxide (CaO) and it was allowed to stand overnight. A dry round-bottomed flask followed by 40-60 ml of ethanol (which was left over night).The mixture was treated until the iodine disappeared. Further 0.5g I_2 was added and heating was continued until all the Mg was converted to ethanolate. Then the remaining amount of ethanol (950ml) was poured into the round-bottomed flask and refluxed for 40 minutes. Then the mixture was distilled and ethanol was collected (b.p 78°C) and stored in well-stopper bottle.

$Mg+2C_2H_5O \rightarrow H_2+Mg\ (OC_2H_5)_2$

$Mg(OC_2H_5)+2H_2O \rightarrow Mg(OH)_2+2C_2H_5OH$

3.3 Experimental Techniques Employed

3.3.1 Chromatographic Technique (Thin layer Chromatography):

Chromatography is a separation technique which depends on the separation is based on differential partitioning between the mobile and stationary phases. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. Thin-layer chromatography (TLC) is a chromatographic technique that is useful for separating organic compounds.

Thin-layer chromatography (TLC) is a chromatographic technique that is useful for separating organic compounds. Thin-layer chromatography can be used to monitor the

progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance. The most commonly used stationary phases, which are available in different grades specially prepared for TLC use; include silica gel, alumina, and cellulose powders.

3.3.2 Thin layer chromatography (TLC)

Ascending one-dimensional thin layer chromatographic technique is used for the initial screening of the extracts and column fractions and checking the purity of isolated compounds. For the latter purpose commercially available pre-coated silica gel (Kiesel gel 60 PF_{254}) plates are usually used. For initial screening, TLC plates are made on glass plates with silica gel (Kiesel gel 60 PF).

Two types of TLC plates were used throughout the experiments.

a) Pre-coated TLC plates: 0.2 mm thin coatings of silica gel on the glass plates or aluminum sheets were used.

b) Manually prepared silica gel coated plates were used.

3.3.3 Preparation of TLC plates

A number of glass plates measuring 20cm x 5cm are thoroughly washed and dried in an oven. The dried plates are then swabbed with acetone-soaked cotton in order to remove any fatty residue. To make the slurry required amount of silica gel 60 PF_{254} and appropriate volume of distilled water (2ml/gm of silica gel) are mixed in a conical flask and the flask is gently shaken. The slurry is then evenly distributed over the plates using TLC spreader. After air drying the coated plates are subjected to activation by heating in an oven at 110^0C for 70 minutes.

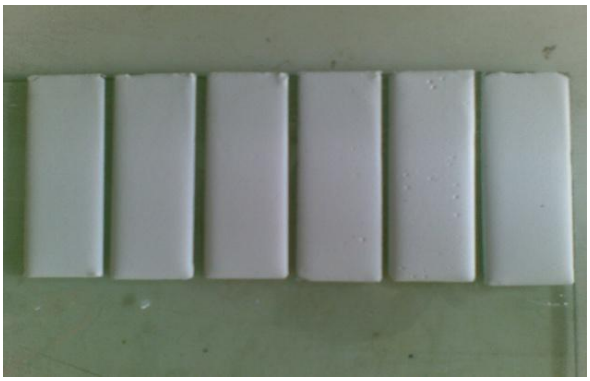


Figure 3.1: Some TLC plates

Table 3.1: Amount of silica gel required preparing TLC plates of various thicknesses

Size (cm × cm)	Thickness (mm)	Amount of silica gel/plate (gm)
20 × 5	0.3	0.9
	0.4	1.2
	0.5	1.5

Cylindrical glass chamber (TLC tank) with airtight lid is used for the development of chromatoplates. The selected solvent system is poured in sufficient quantity into the tank. A smooth sheet of filter paper is introduced into the tank and allowed to soak in the solvent. The tank is then made airtight and kept for few minutes to saturate the internal atmosphere with the solvent vapor.

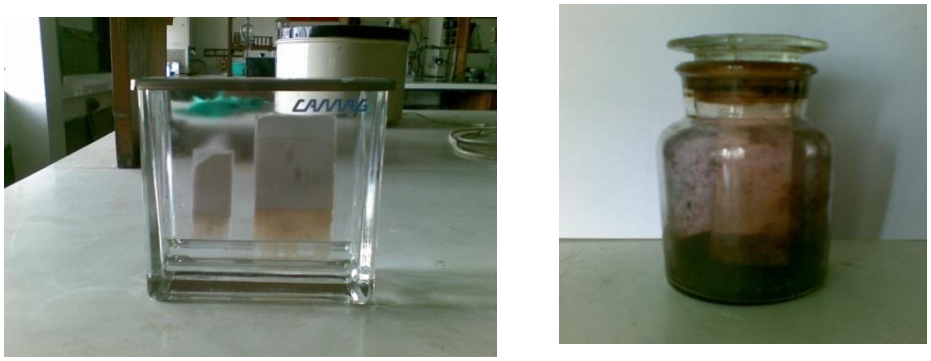


Figure 3.2: TLC tank & iodine chamber

A small amount of dried extract is dissolved in a suitable solvent to get a solution (approximately 1%). A small spot of the solution is applied on the activated silica plate with a capillary tube just 1 cm above the lower edge of the plate. The spot is dried with a hot air blower and a straight line is drawn 2 cm below the upper edge of the activated plate which marks the upper limit of the solvent flow. The spotted plate is then placed in the tank in such a way as to keep the applied spot above the surface of the solvent system and the cap/lid is placed again. The plate is left for development. When the solvent front reaches up to the given mark, the plate is taken out and air-dried. The properly developed plates are viewed under UV light of various wavelengths (254 nm and 366 nm) as well as treated with suitable reagents to detect the compounds.

3.3.4 Sample application (Spotting the plates)

The TLC and PTLC plates were spotted with a small amount of the crude extract by using a narrow glass capillary. The capillary must be washed in a solvent (acetone) before each sample was applied.

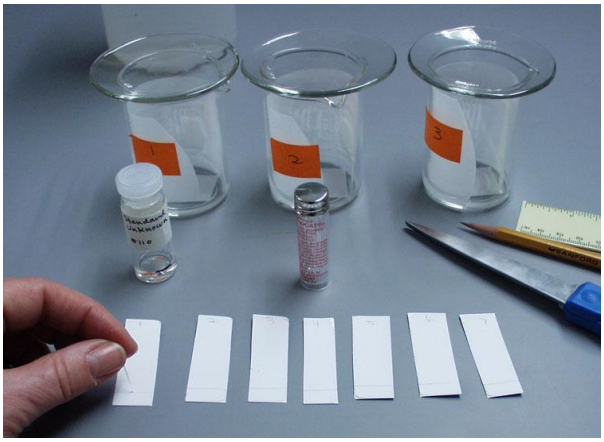


Figure 3.3: process of spotting

3.3.5 Solvent treatment

Solvent treatment is a process by which a compound consisting of the major portion of a mixture of compounds can be purified utilizing selective solvent washing. Initially, a solvent or a solvent mixture in which the desired compound is practically insoluble and

other components are soluble is chosen. The undesired components are separated with repeated washing with this solvent or solvent mixture. If required other solvent or solvent mixture can be used until a pure compound is obtained.

3.3.6 Developments of Plates

When the sample was spotted on the film of plated, it was developed in ascending process. Usually two plates were placed in one tank. The plates were put in the tank in such a way that they were inclined and the lower edge immersed in the selected mobile phase, but the solvent barrier was somewhat below than the starting line.

3.3.7 Visualization/detection of compounds

Detection of compounds in TLC plates is a very important topic in analyzing extractives to isolate pure compounds. The following techniques are used for detecting the compounds in TLC/PTLC plates.

3.3.8 Iodine chamber

The developed chromatogram is placed in a closed chamber containing crystals of iodine and kept for few minutes. The compounds that appeared as brown spots are marked. Unsaturated compounds absorb iodine. Bound iodine is removed from the plate by air blowing.

3.3.9 Determination of R_f (retardation factor) values

R_f value is characteristic of a compound in a specific solvent system. It helps in the identification of compounds. R_f value of a compound can be calculated by the following formula:

$$R_f = \frac{\text{Distance (cm) traveled by solute}}{\text{Distance (cm) traveled by solvent}}$$

Usually, the R_f value is constant for any given compound and it corresponds to a physical property of that compound.

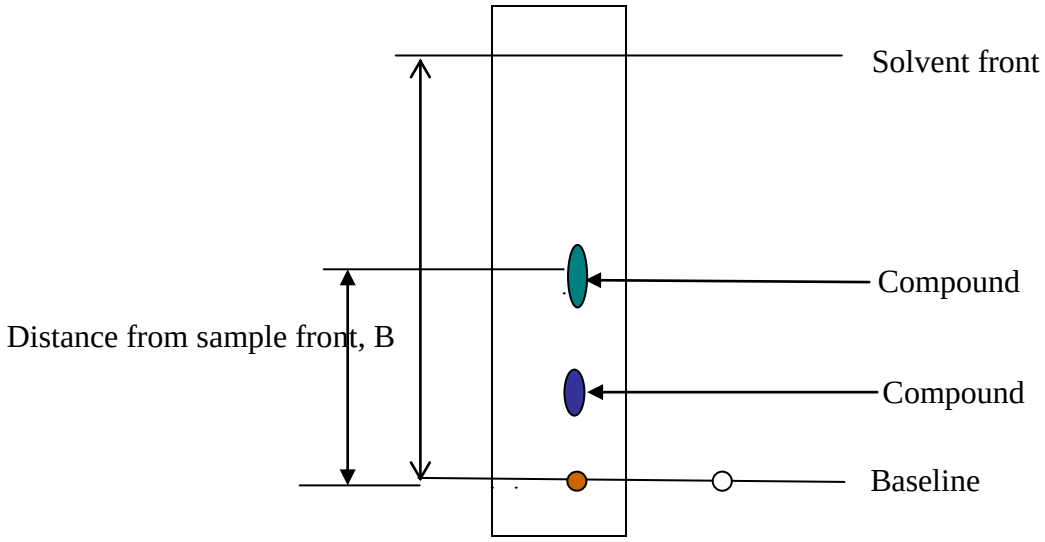


Figure 3.4: A Plate for the calculation of R_f value

3.3.10 Rerystallization

Crystallization was employed as a final purification process. The solvent in which the compound was dissolved in a minimum volume of solvent in hot condition and was left undisturbed for crystallization. Sometimes mixtures of solvents were used. The compound was dissolved in a suitable solvent and then a solvent in which the compound was insoluble, was gradually until cloudiness developed in the solution.

3.4 Spectroscopic techniques

a) Infra-red (IR) spectroscopy

A Shimadzu IR-470 spectrometer was used to record the infra-red spectrum (KBr pellet). Before making the pellets, the samples were dried in the desiccator to remove moisture completely and to avoid unexpected O-H peak for accumulated water molecules in the sample.

b) ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy

NMR spectra of the samples were recorded on a 500 MHz NMR spectrometer. The solvents used were DMSO and $CDCl_3$. TMS was used as an internal standard.

Abbreviation Used

UV	Ultraviolet
IR	Infra-red
NMR	Nuclear magnetic resonance
S	Singlet
Bs	Broad single
D	Doublet
J	Coupling constant
TLC	Thin Layer Chromatography
R_f	Retarding factor
Bp	Boiling point
Hz	Hertz
TMS	Tetra methyl Silane

c) Melting point apparatus

Melting point (m.p) was determined by using an electro thermal melting point apparatus (Mel-temp, OGAWA SEIKICO, and Japan).

Figure 3.5 : Melting point apparatus

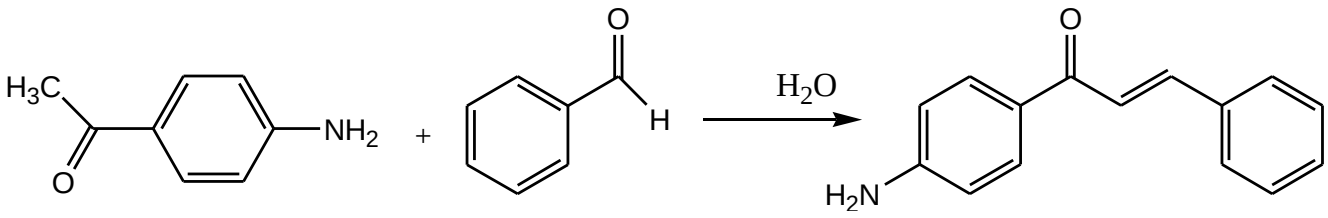
3.5 Preparation of Substituted Chalcones

3.5.1 Preparation of 1-(4-amino-phenyl)-3-phenyl-propenone (R.P-1 compound)

Procedure

4-amino acetophenone (0.01mol) and benzaldehyde (0.01mol) was dissolved in 100ml ethanol. After 40 min, to this solution, NaOH (40%, 10ml) was added drop wise with constant. The rate of addition was adjusted so that the temperature remains 25-30c. The reaction mixture was kept 23-24 hr. The progress of the reaction and formation of the product will be monitored by TLC on silica gel plates (eluting solvents, ethylacetate: hexane, 60:40). The R_f value of the product was 0.45. When both reactants disappeared, the reaction mixture was neutralized by 0.01M HCl Where by precipitation occurred. The precipitate was filtered off and washed with cold water until the washings were neutral to litmas. It was crystallized from ethanol.

Reaction Scheme



4-amino acetophenone Benzaldehyde 1-(4-Amino-phenyl)-3-phenyl-propenone

Characterization and structure determination of the product

The structure determination of the product was 1-(4-amino-phenyl)-3-phenyl-propenone given Fig 3.6 The product of the color was yellow color and the melting point of the pure product was 142°-143°C.

Spectroscopic characteristic for R.P-1 compound

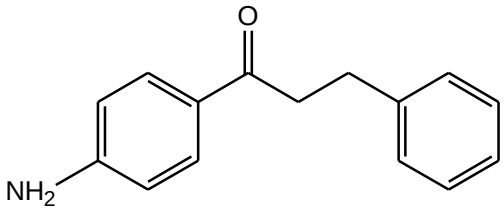
(i) IR spectrum

The IR spectrum of the product (3.6) run as KBR pellet showed absorption band ν_{max} in cm^{-1} which as assigned as: 3362, 3000, 1652, and 1229.

(ii) ^{1}H-NMR spectrum (400 MHz, in $CDCl_3$)

The ^{1}H-NMR spectrum of the compound (RP-1) was taken in $CDCl_3$ and TMS was used as reference showed 5.067 (NH_2), 6.595-6.742 (m, 2H, Ar), 7.332-7.587 (m, 5H, Ar), 7.608-7.789 (m, 3H) , and 7.962 (d, *J* = 8.8 Hz, 1H).

On the basis of physical properties, chemical behavior and spectral properties the following Fig (3.6) has been assigned to this product.



1-(4-amino-phenyl)-3-phenyl-propenone
Figure: 3.6 (Compound I)

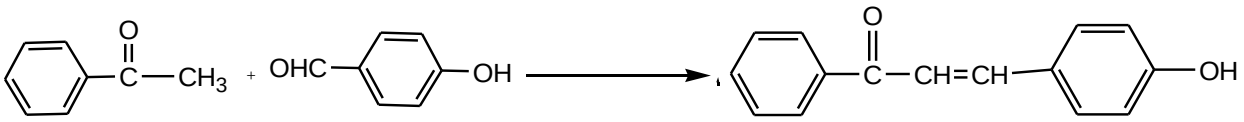
3.5.2 Preparation of 3-(4-hydroxy-phenyl)-1 phenyl-propenone

Procedure

4-hydroxyl benzaldehyde (0.01mol) and acetophenone (0.01mol) was dissolved in 100 ml ethanol. After 40 min, to this solution, NaOH (40%, 10 ml) was added drop wise with constant stirring .The rate of addition was adjusted so that the temperature remains 25-30°c. The reaction mixture was kept 23-24 hrs. .The progress of the reaction and formation of the product was monitored by TLC on silica gel plates (eluting solvents, ethylacetate: hexane, 60:40). The R_r value of the product was 0.30. When both reactants disappeared,

the reaction mixture was neutralized by 0.01M HCl Where by precipitation occurred. The precipitates were filtered off and wash with cold water until the washings were neutral to litmus. It was crystallized from ethanol. The melting point of the pureproduct was 183-184^0 C. The yield of the pure 3-(4-aminophenyl)-3-phenyl propenone was 9.4g.

Reaction scheme:



Acetophenone 4-hydroxy benzaldehyde 3 (4-hydroxy phenyl) -1-phenyl -propenone

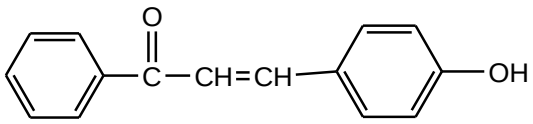
Characterization and structure determination of the product

The structure determination of the product was 3-(4-hydroxy-phenyl)-1 phenyl-propenone given Fig (3.7) the product of the color was orange yellow color. The melting point of the pure product was 183-184 ºC.

IR Spectrum

The IR spectrum of the product Fig (3.7) run as KBR pellet showed absorption band V_{max} in cm^{-1} which as assigned as: 3268, 3050, 1650, 1602, 936, and v836.

On the basis of chemical behavior and spectral properties the following Fig (3.7) has been assigned to this product



3 (4-hydroxy phenyl) -1-phenyl -propenone

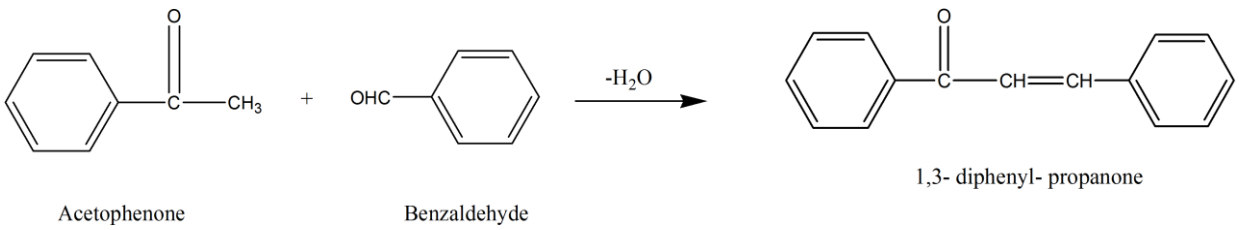
Figure: 3.7

3.5.3 Preparation of 1, 3- diphenyl-propanone

Procedure

Acetophenone (3.60 g) and Benzaldehyde (3.18 g) were dissolved in rectified spirit (50 ml) in a round bottomed flask, equipped with a magnetic stirrer. The reaction vessel was immersed in a bath of cold water and aqueous sodium hydroxide solution (5g NaOH in 50 ml water) was added to the mixture drop wise during 30 minutes. The rate of addition was adjusted so that the temperature remained between 20-25°C. The progress of the reaction was followed by TLC silica gel plates (eluting solvent; chloroform). The R_f value of the product was 0.50. The stirring was continued for 6 hours at 20-25°c. When both reactants disappeared, the reaction mixture was neutralized by 0.1N HCl where by precipitation occurred. The precipitation was filtered off and washed with cold water until the washings were neutral to litmus and then with 20 ml of ice cold rectified sprit. The crude product was recrystallized from hot rectified spirit and filtered, washed with cold rectified spirit and dried.

Reaction scheme



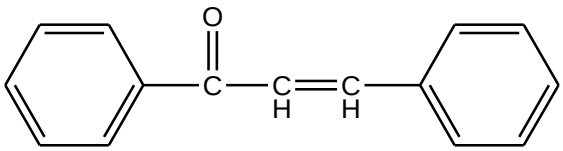
Characterization and structure determination of the product

The structure determination of the product was Preparation of 1, 3-diphenyl-propanone given Fig (3.8). The product of the color was yellow color and melting point 46-47ºC.

IR Spectrum

The IR spectrum of the product Fig (3.8) run as KBR pellet showed absorption band ν_{max} in cm^{-1} which as assigned as: 3059, 2926, 1661, 1603, 1447, 1217, and 746.

On the basis of chemical behavior and spectral properties the following Fig (3.8) has been assigned to this product.

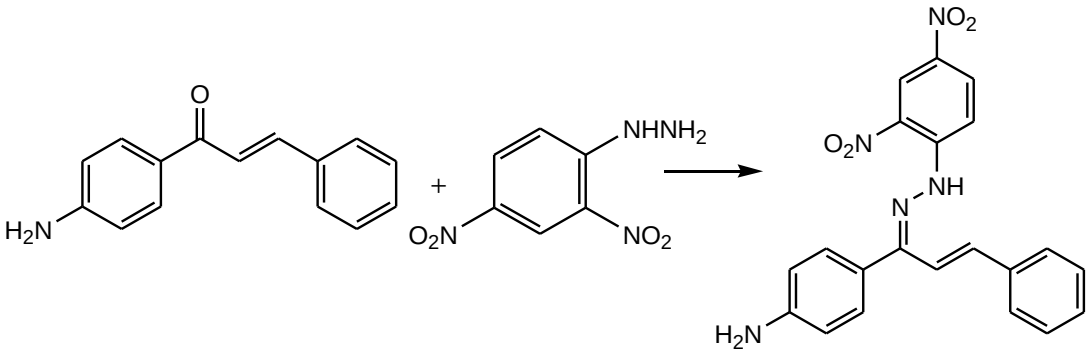


1,3-diphenyl-propenone
Figure: 3.8

3.6 Preparation of 4-{1-[(2, 4-Dinitro-phenyl)-hydrazono]-3-phenyl-allyl} phenylamine (compound II)

1-(4-amino-phenyl)-3-phenyl-propenone (0.01mol) was dissolved in 100ml ethanol. Then 2,4 di nitro phenyl hydrazine was added drop wise with constant stiring. The rate of addition was adjusted so that the temperature remains 25-30c. The reaction mixture was kept 0.5 hr. The progress of the reaction and formation of the product will be monitored by TLC on silica gel plates (eluting solvents, ethylacetate: hexane, 60:40). The R_f value of the product was 0.65.At the end of this reaction precipitation occured. The precipitate was filtered off and dried m.p. at 206-208ºC.

Reaction Scheme



Product-1 2,4 dinitrophenylhydrazine Product-2

Spectroscopic characteristic for R.P-3 compound

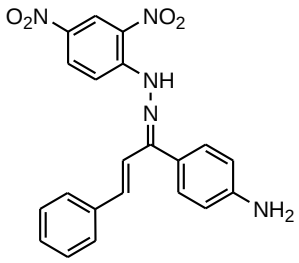
(i) IR spectrum

The IR spectrum of the product Fig (3.9 R.P-3) run as KBR pellet showed absorption band ν_{max} in cm^{-1} which as assigned as: 3286-3444, 1658, and 1618, 1360-1528, ad 1133-1137.

(ii) NMR spectrum (400 MHz, $CDCl_3$)

The ^{1}H-NMR spectrum (Fig. 4.6) of the compound Fig (3.9 R.P-3) was taken in $CDCl_3$ and TMS was used as reference showed the value of 1.275 (s, NH_2), 7.084 (s, NH), 7.489-7.505 (m, 5H), 7.794-7.818 (m, 3H), 8.124-8.164 (m, 3H, Ar), 8.394 (d, J = 12 Hz, 2H, Ar), 9.185 (d, *J*= 2.4Hz, 1H, Ar), and 11.350 (s, 1H, Ar).

On the basis of chemical behavior and spectral properties the following Fig (3.9 R.P-3) has been assigned to this product



4-{1-[(2,4-Dinitro-phenyl)-hydrazono]-3-phenyl-allyl}-phenylamine

Figure: 3.9 (Compound II)

3.7 Reaction of arylideneacetophenone with pyrimidine derivative (Compound III)

Procedure

Synthesis can be done in two ways such as

(a) Microwave Irradiation Method

(b) Oil Bath Method

(a) Microwave Irradiation Method

A mixture of barbituric (0.77g, 0.006 mol) and chalcone (1.11g, 0.005mol) was taken in a round bottom flask (250). To this solution aqueous ethanol (32ml) was added. The mixture was then reflux for 5-7 minutes with constant stirring at 105ºC. The progress of the reaction reaction was followed by TLC on silica plates (eluting solvent ethyl acetate:hexane). This reaction product was obtained but few amount of reactant was stay.

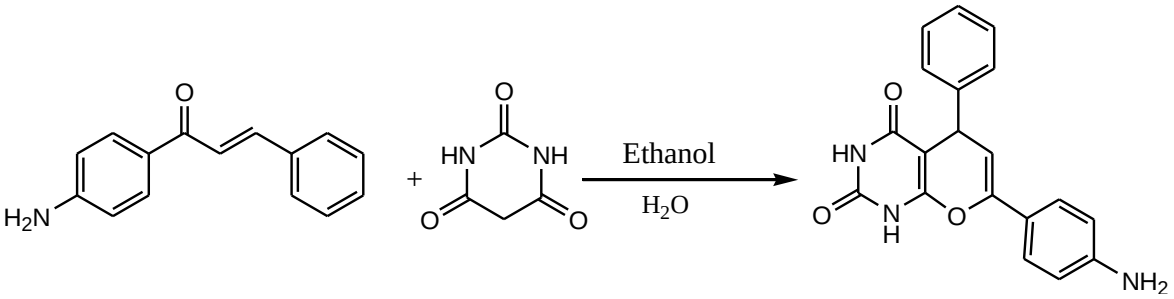
(b) Oil Bath Method

A mixture of barbituric (0.35g, 0.0025 mol) and chalcon (0.56g, 0.0025mol) was taken in a round bottom flask (250) The reaction mixture was refluxed. Thus the progress of reactions was followed by TLC on silica gel plate (eluting solvent ethyl acetate: n-hexane; 90:10). The R_f value of the product was 0.37. The stirring was continued for 72 hours with constant stirring at temperature 105ºC. When both reactants disappeared, the precepitation of the reaction was filtered off and washed with cold water. The crude product was recrystallized from rectified spirit and dried. The purity of the product was checked by TLC from ethyl acetate n-hexane and (90:10). It was crystallized from ethanol. The melting point of the pure product was 133°-134°C.

Characterization and structure determination of the product

The structure determination of the product was 7-(4-Amino-phenyl)-5-benzyl-1,5-dihydro-pyrano[2,3-d]pyrimidine-2,4-dione given Fig (3.10 R.P-4) The product of the color was yellow color and melting point 133-134ºC.

Reaction Scheme



Product (1) Barbituric acid Pyrimidine Derivatives Final Product

Spectroscopic characteristic for R.P-2 compound

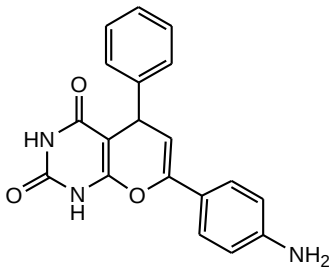
(i) IR spectrum

The IR spectrum of the product Fig (3.10) run as KBR pellet showed absorption band ν_{max} in cm^{-1} which as assigned as: 3482-3430, 3000, 1698 ,1602, and 1367.

(ii) NMR spectrum (400 MHz, in DMSO)

The ^{1}H-NMR spectrum of the compound Fig (3.10 R.P-2) was taken in $CDCl_3$ and TMS was used as reference showed the value of 3.70-3.90 (s, 1H, NH_2), 6.00-6.20 (m, 1 H, NH), 6.50-6.60 (m, 2H, Ar), 7.10-7.80 (m, 7H, Ar), and 10.90-11.20 (m, 1H, NH).

On the basis of chemical behavior and spectral properties the following Fig (3.10 R.P-2) has been assigned to this product.



7-(4-Amino-phenyl)-5-phenyl-1, 5-dihydro-pyrano [2, 3-d] pyrimidine-2, 4-Dione

Figure**:** 3.10 (Compound III)

3.8 Antimicrobial Activity

3.8.1 Introduction

The increasing clinical incidences of drug resistant and bacterial pathogens have attracted additional urgency to antimicrobial drug research. The antimicrobial screening which is the first stage of antimicrobial drug research is performed to ascertain the susceptibility of various bacteria to any agent. This test measures the ability of each test sample to inhibit the in vitro bacterial and growth. This test measures the ability of each test sample to inhibit the in vitro fungal and bacterial growth. This ability may be estimated by any of the following three methods.

- Disc diffusion method
- Broth microdilution
- Broth macrodilution
- Agar dilution Agar dilution

The antibacterial activity of all the synthesized compounds was examined against three gram positive organisms and three gram negative organisms. Antimicrobial and antifungal screening of all the compounds was done by using disc diffusion method at a concentration level of 12µg/µL and 300µg/disc. Ciprofloxacin and miconazol were used as standard drug at a concentration level of 25µg/disc and 50µg/disc respectively for antimicrobial and antifungal test. Activity of the compounds were recorded by measuring the zone of inhibition in mm, and compared with the standard zone of inhibition produced by Ciprofloxacin and miconazol. This determination indicates whether the organism is sensitive or resistant to the synthesized compounds.

3.8.2 Principle of Disc Diffusion Method

The disc diffusion method allows for the simultaneous testing of a large number of antimicrobials in a relatively easy and flexible manner. In this method, the bacterial inoculum is adjusted to certain concentration, inoculated onto the entire surface of a Mueller-Hinton agar (MHA) plate with a sterile cotton-tipped swab to form an even lawn. The paper disks (6 min in diameter; BD Diagnostic System) impregnated with diluted

antibiotic solution was placed on surface of each MHA plate using a sterile pair of forceps. Then the plates ere incubated aerobically and the diameter of zone of inhibition was measured by a ruler or caliper. Based on the diameter of the inhibition zone and, the results are then assigned to three categories, susceptible, intermediate, or resistant. The bigger the diameter of the inhibition zone, the more susceptible is the microorganism to the antimicrobial [84].

In the present study all the samples were tested for antimicrobial activity by disc diffusion method.

3.8.3 Test materials of pyrimidine derivatives

7) 1-(4-Amino-phenyl)-3-phenyl-propenone
8) 7-(4-Amino-phenyl)-5-phenyl-1,5-dihydro-pyrano [2, 3-d] pyrimidine-2, 4-Dione and
9) 4-{1-[(2, 4-Dinitro-phenyl)-hydrazono]-3-phenyl-allyl}-phenylamine

3.8.4 Test Organisms

The bacterial and fungal strains used for the experiment were collected as pure cultures. Antibacterial activity was determined against three gram-positive bacteria (*Bacillus cereus, Staphylococcus aureus* and *Listeria monocytogenes*) and three gram-negative bacteria (*Escherichia coli, Salmonella typhimurium, and citobacteria frundi*). Antifungal screening was carried out against one fungi (*Tricodarma harzianum*).

Gram positive Bacteria	**Gram negative Bacteria**	**Fungi**
Bacillus cereus	*Escherichia coli*	*Tricodarma harzianum*
Staphylococcus aureus	*Salmonella typhimurium*	
Listeria monocytogenes	*Citobacter freundi*	

Table 3.2: List of Test Bacteria and fungi

3.9 Methods

3.9.1 Determination of antimicrobial activity by the zone of inhibition

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

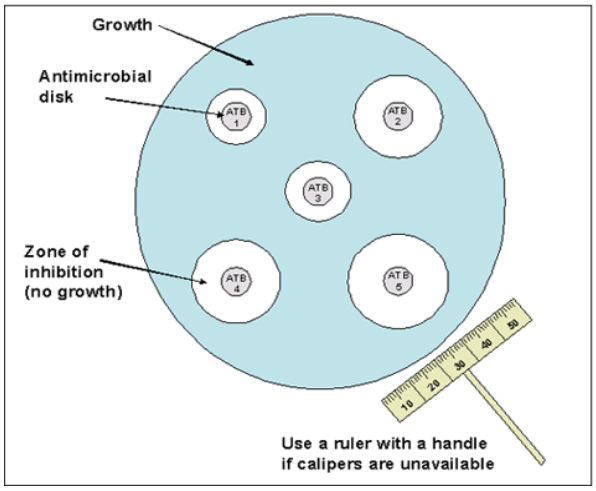


Figure 3.11: Inhibition Zone measurement

3.9.2 Disc diffusion methods

The Kirby-Bauer and Stokes' methods are usually used for antimicrobial susceptibility testing, with the Kirby-Bauer method being recommended by the National Council of Clinical Laboratory Service (NCCLS). The accuracy and reproducibility of this test are dependent on maintaining a standard set of procedures as described here.

Materials used

- Test Organisms: Three gram positive organisms, three gram negative organisms and one fungus were used for the determination of activity

- Growth Media: The activity was conducted on the Nutrient Agar Media produced from TSA (Tryptone Soya Agar).

Apparatus used

- Petri plate : Plastic plate, which was previously sterilized
- Pipette: Micropipette was used for adding the required concentration of sample to the plates.
- Blank discs : Susceptible blank discs were used, which was stored in -20^0C to 8^0C.
- Glasswares : 500 mL conical flask and test tubes were used.
- Compounds Screened : All the synthesized compounds.
- Solvent Used : Dimethyl sulfoxide.
- Standard Used : Ciprofloxacin acid.

Procedure for performing the disc diffusion test

Inoculum preparation

Growth method

The growth method is performed as follows

a) At least three to five well-isolated colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a loop, and the growth was transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth.
b) The broth culture was incubated at 37°C until it achieved or exceed the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours).

c) The turbidity of the actively growing broth culture was adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard.

Inoculation of test plates for bacteria

a) Media was prepared by adding 40.0 gm of Nutrient agar to 1L of distilled water. Then it was sterilized by autoclaving at 15 lb/inch and at 121^0C temperatures for two hours.
b) Media was cooled to the temperature of approximately 40^0C 25mL was transferred to a Petri plates. Plates were allowed to cool for 20 minutes.

c) The plates were incubated for 24 hours at 37°C and checked for any contamination.

d) The dried surface of a fresh TSA plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed.
e) The lid may be left the plate for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

Inoculation of test plates for fungi

a) Media was prepared by adding 40.0 gm of Nutrient agar to 1L of distilled water. Then it was sterilized by autoclaving at 15 lb/inch and at 121^0C temperatures for two hours.
b) Media was cooled to the temperature of approximately 40^0C. 25mL was transferred to a Petri plates. Plates were allowed to cool for 20 minutes.

c) The plates were incubated for 24 hours at 30°C and checked for any contamination.

d) The dried surface of a fresh SDA plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed.

e) The lid may be left the plate for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

Figure 3.12: Swabbing of Test plates

Application of discs to inoculated agar plates

a) The predetermined battery of antimicrobial discs was dispensed onto the surface of the inoculated agar plate. Each disc was pressed down to ensure complete contact with the agar surface. The discs were placed such a way so that they were no closer than 24 mm from center to center.
b) The TSA and SDA plates are inverted and placed in an incubator set to 37°C and 30°C within 15 minutes after the discs were applied in shown in figure 3.8.

Figure 3.13: Application of discs

3.10 Application of samples on the discs

a) Compounds 7-8 were dissolved in DMSO and diluted to get a concentration of 12 µg/µL.
b) Six blank discs were placed in the petri plates. Reference standard ciprofloxacin and miconazol was impregnated on one of the discs, and only solvent as a blank was impregnated on one of the discs, and others experimental solutions were impregnated on others discs. Each disc's was marked by a marker as a small symbol so that each of the discs could be easily identified. 25µl of solution was injected on each disc.

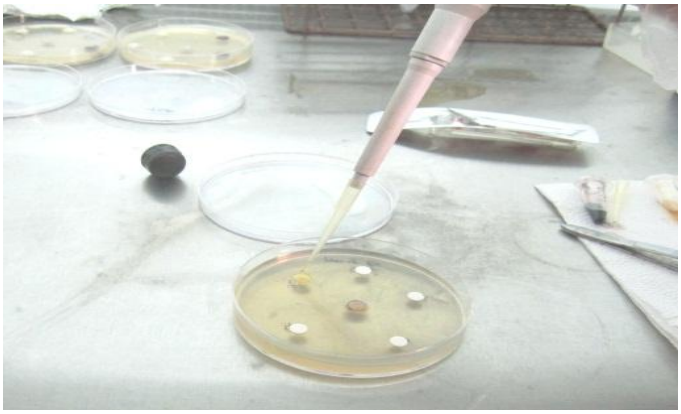


Figure 3.14: Application of Samples on the discs

CHAPTER IV

RESULTS AND DISCUSSIONS

CHAPTER V

Conclusions

In conclusion, a new class of chalcone and pyrimidines derivatives were synthesized and evaluated as antimicrobial activity. The synthesis of chalcone was carried out by1-(4-Amino-phenyl)-3-phenyl-propenone (**I**) with 2,4 dinitrophenyl hydrazine and pyrimidine derivative (Barbituric acid). The formed chalcone with treated with barbituric acid by refluxing condensation using ethanol to give 7-(4-Amino-phenyl)-5-phenyl-1,5-dihydro-pyrano[2,3-d]pyrimidine-2,4-Dione (**III**) and also treated with 2,4 dinitrophenyl hydrazine to gave{4-{1-[(2,4-Dinitro-phenyl)-hydrazono]-3-phenyl-allyl}-phenylamine}(**II**). The structural elucidation of synthesized compounds were synthesized successfully and characterized by IR and ^{1}NMR. All the synthesized compound were subject to antibacterial and antifungal activity. The antibacterial activity of the compounds at concentration at 300µg/disc was performed against three gram-positive bacteria (*Bacillus cereus, Staphylococcus aureus* and *Listeria monocytogenes*) and three gram-negative bacteria (*Escherichia coli, Salmonella typhimurium, and citobacteria frundi*) by Kirby-Bauer disc diffusion method using Ciprofloxacin as standard. Antifungal screening was carried out against one fungi (*Tricodarma harzianum*) using Michonazole as standard. From the data it is observed that Compound **I** {1-(4-Amino-phenyl)-3-phenyl-propenone} exhibits no activity against the tested bacterial strains but showed moderate activity against the tested fungi *Trichoderma harziniaum. Ctirobacter freundi* was found to be resistant for most of the compound. Compound **III** {7-(4-Amino-phenyl)-5-phenyl-1,5-dihydro-pyrano[2,3-d]pyrimidine-2,4-Dione} exhibits broad spectrum activity against both gram negative and gram positive strains tested except *Listeria monocytogenes*. Also the compound 9a showed promising antibacterial activity against *Ctirobacteria freundi* due to their presence of nitro and hydrazine group. Compound **II**; {4-{1-[(2,4-Dinitro-phenyl)-hydrazono]-3-phenyl-allyl}-phenylamine} which was synthesized from compound 7a showed moderate antibacterial activities against *citobacteria frundi* . Thus due to the presence of reactive amino group, nitro group, hydrazine group and unsaturated ketone function in synthesized compounds may be responsible for their potential antimicrobial and antifungal activity.

CHAPTER V

CONCLUSION

&

RECOMMENDATIONS

CHAPTER IV

Results and Discussion

4.1 Reaction of 4-aminoacetophenone with benzaldehyde

The reaction of 4-aminoacetophenone with benzaldehyde, ethanol and water at refluxing temperature gave a yellow crystalline solid, m.p. 142 -143°C. The R_f value of the product was found to be 0.45 on TLC [Ethyl acetate: n- hexane= 60:40].

Spectral Properties

The IR spectrum (Fig 4.1) of the product run as KBr pellet showed the following absorption bands, ν_{max} in cm^{-1}.

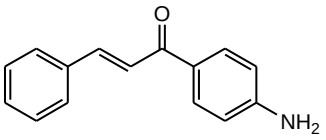
Absorption bands, ν_{max} in cm^{-1}	Group presence
3362-3374	-NH_2 stretching
3000	C-H aromatic proton
1652	C=O in conjugation
1602	C=C in conjugation
1229	C-N stretching

Table 4.1: IR value of the compound R.P-1

The ^1H NMR spectrum (Fig: 4.2) of the compound in $CDCl_3$ gave the following signals (δ value) using TMS as an internal standard.

5.067 (NH_2), 6.595-6.742 (m, 2H, Ar), 7.332-7.587 (m, 5H, Ar), 7.608-7.789 (m, 3H) , 7.962 (d, *J* = 8.8 Hz, 1H)

On the basis on the properties (IR, NMR) and chemical behavior, structure (Compound-1) has been assigned to the obtained product.



1-(4-Amino-phenyl)-3-phenyl-propenone (Compound-1)

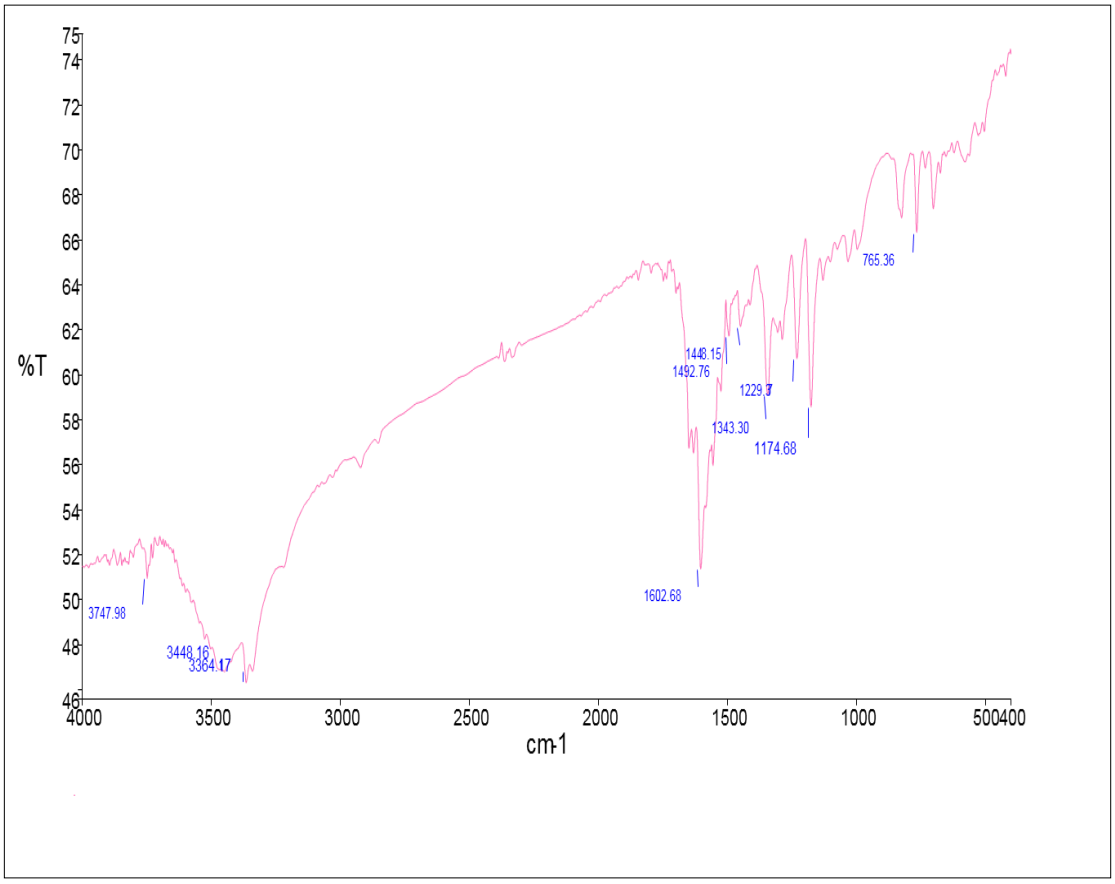


Figure 4.1: IR spectrum of R.P-1 compound

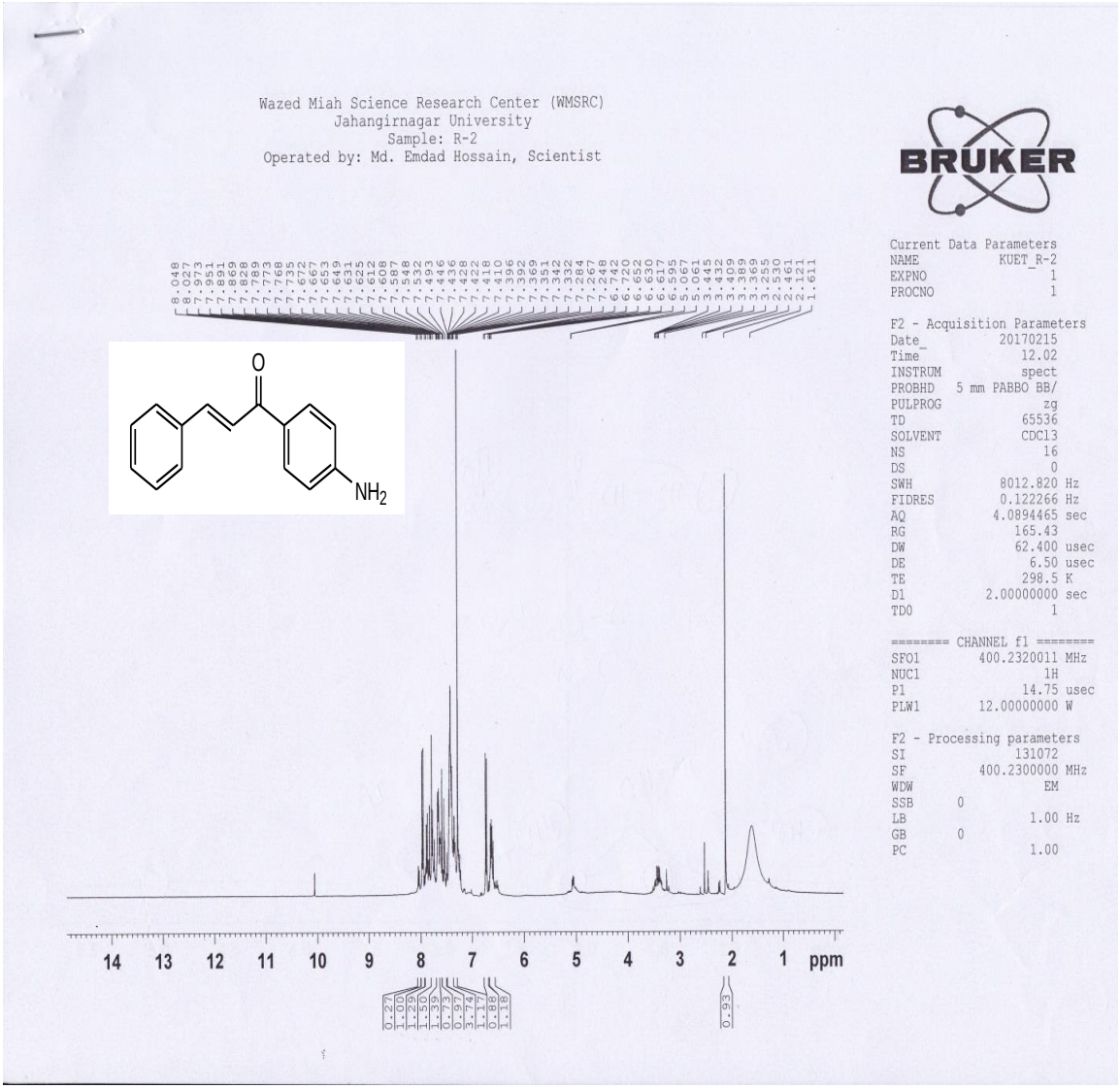


Figure 4.2: NMR spectrum of the compound compound-I

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4.2 Reaction of 1-(4-Amino-phenyl)-3-phenyl-propenone with barbituric acid

The reaction of 1-(4-Amino-phenyl)-3-phenyl-propenone with barbituric acid in ethanol and water at refluxing temperature gave a yellow crystalline solid, m.p. 133 -134°C. The R_f value of the product was found to be 0.37. On TLC [Ethyl acetate: n- hexane].

Spectral Properties

The IR spectrum (Fig: 4.3) of the product run as KBr pellet showed the following absorption bands, ν_{max} in cm^{-1}.

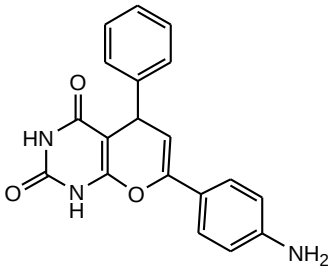
Absorption bands, ν_{max} in cm^{-1}	Group presence
3482-3430	$-NH_2$ and -NH- stretching
3000	C-H aromatic proton
1698	C=O in conjugation
1602	C=C in conjugation
1367	C-N stretching

Table 4.2: IR value of the compound R.P-2

The ^1H NMR spectrum (Fig: 4.4) of the compound in DMSO gave the following signals (δ value) using TMS as an internal standard.

3.70-3.90 (s, 1H, NH_2), 6.00-6.20 (m, 1 H, NH), 6.50-6.60 (m, 2H, Ar), 7.10-7.80 (m, 7H, Ar), 10.90-11.20 (m, 1H, NH)

On the basis on the properties (IR, NMR) and chemical behavior, structure (compound-2) has been assigned to the obtained product.



7-(4-Amino-phenyl)-5-phenyl-1, 5-dihydro-pyrano [2, 3-d] pyrimidine-2, 4-Dione

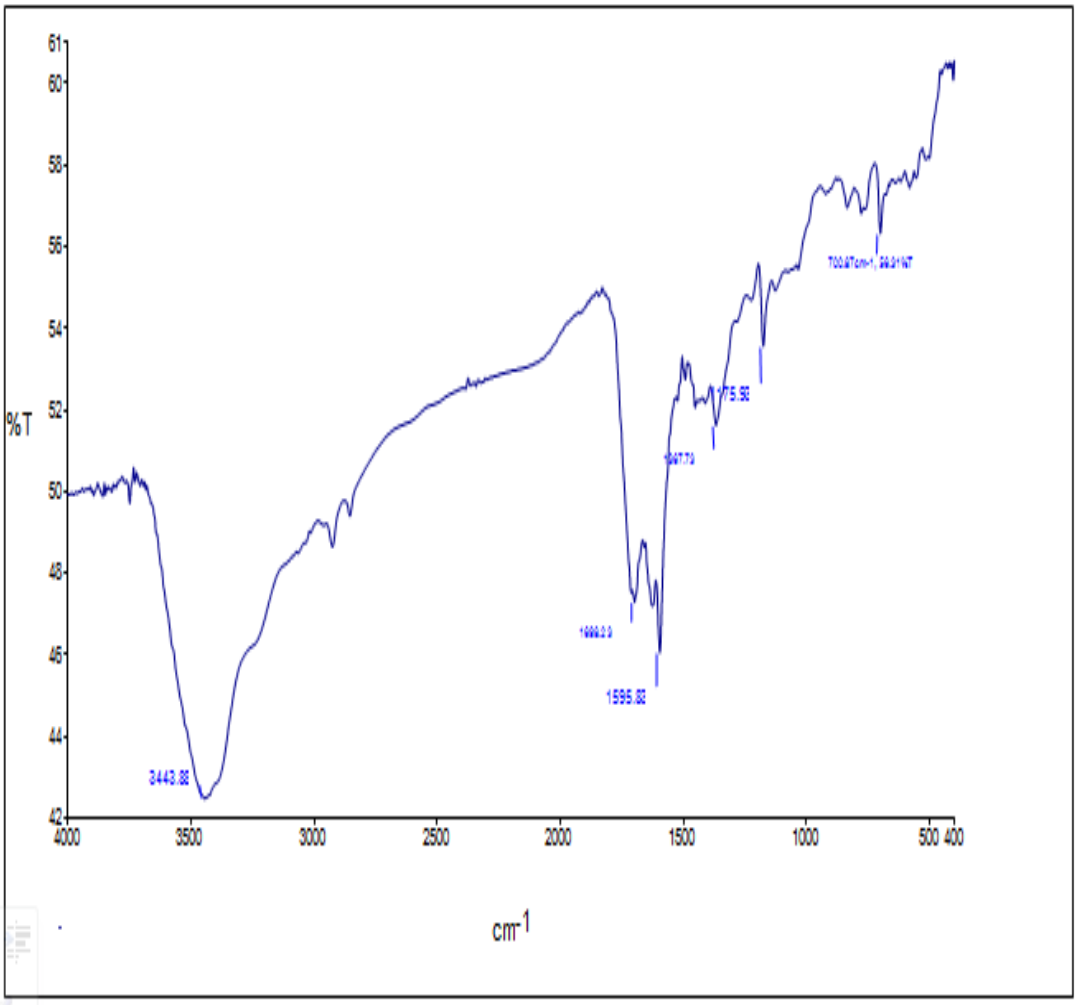


Figure 4.3: IR spectrum of compound II

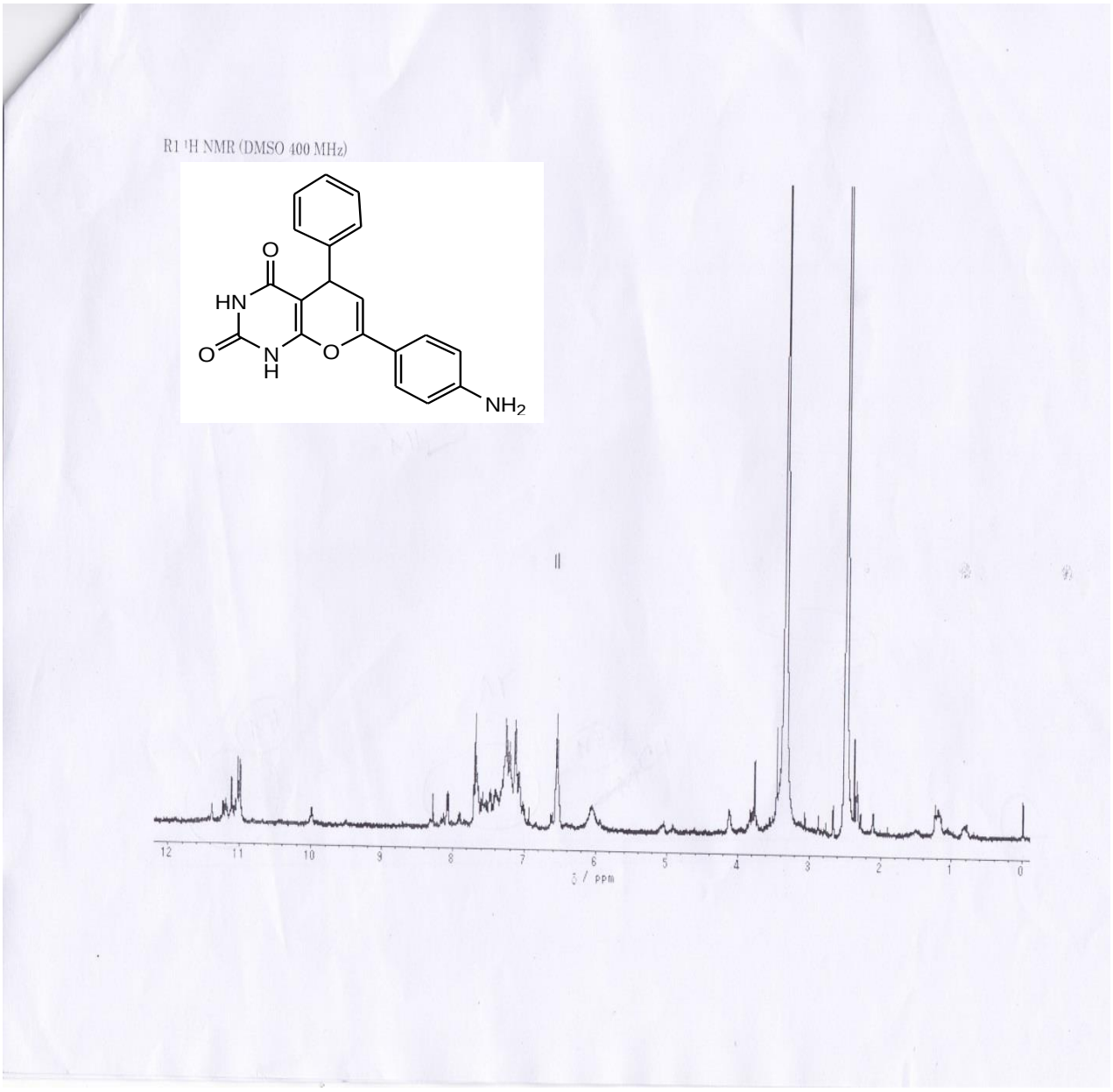


Figure 4.4: NMR spectrum of the R.P-2 compound in DMSO

4.3 Reaction of 1-(4-Amino-phenyl)-3-phenyl-propenone with 2,4dinitrophenyl hydrazine

The reaction of 1-(4-Amino-phenyl)-3-phenyl-propenone with 2, 4 dinitrophenylhydrazine at room temperature was given an orange crystalline solid, m.p. at 206 -208°C.

Spectral Properties

The IR spectrum (Fig: 4.5) of the product run as KBr pellet showed the following stretching bands, ν_{max} in cm^{-1}.

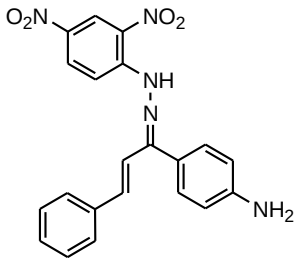
Absorption bands, ν_{max} in cm^{-1}	Group presence
3286-3444	$-NH_2$ and -NH- stretching
1360-1528	$-NO_2$ stretching
1658	C=O in conjugation
1618	C=C in conjugation
1133-1337	C-N stretching

Table 4.3: IR value of the compound R.P-3

The ^1H NMR spectrum (Fig: 4.6) of the compound in $CDCl_3$ gave the following signals (δ value) using TMS as an internal standard.

1.275 (s, NH_2), 7.489-7.505 (m, 5H), 7.794-7.818 (m, 3H), 8.124-8.164 (m, 3H, Ar), 8.394 (d, J = 12 Hz, 2H, Ar), 9.185 (d, *J*= 2.4Hz, 1H, Ar), 11.350 (s, 1H, Ar), 11.350 (s, 1H, NH),

On the basis on the properties (IR, NMR) and chemical behavior, structure (IV-R-3) has been assigned to the obtained product.



4-{1-[(2, 4-Dinitro-phenyl)-hydrazono]-3-phenyl-allyl}-phenylamine (R.P-3)

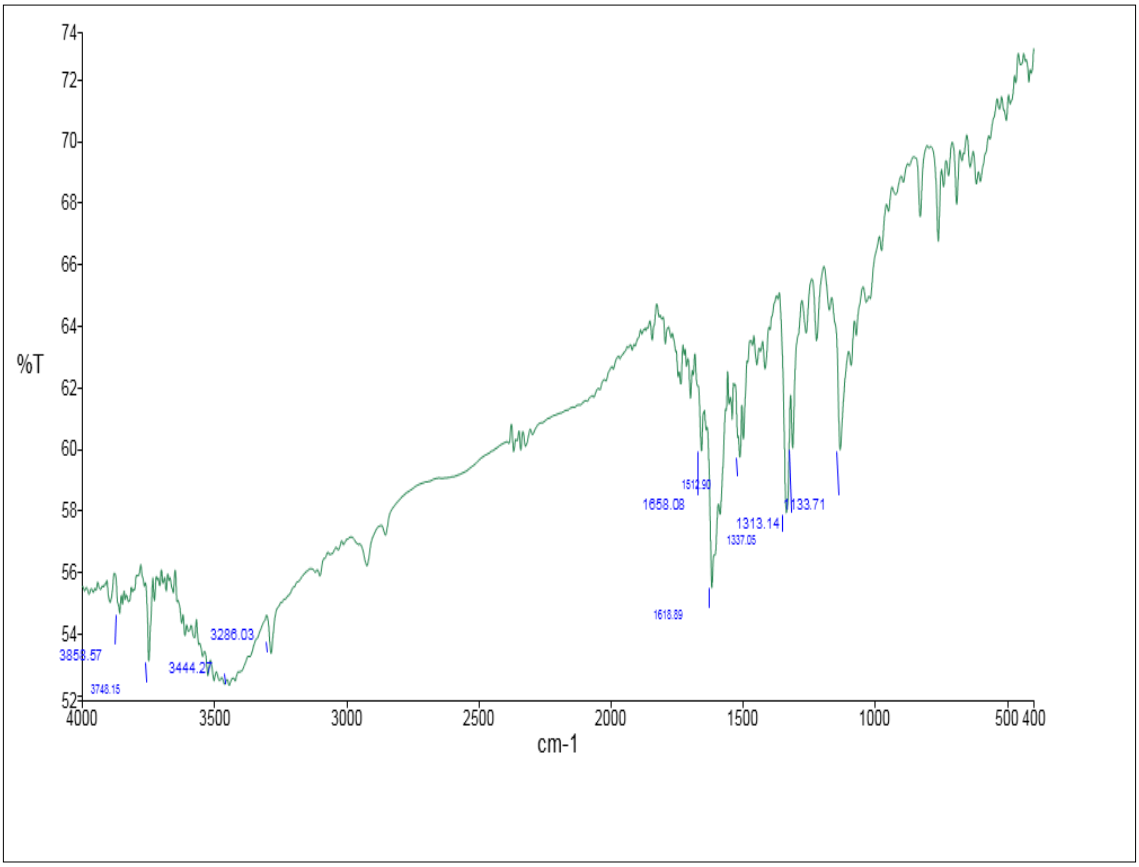


Figure 4.5: IR spectrum of R.P-3 compound in $CDCl_3$

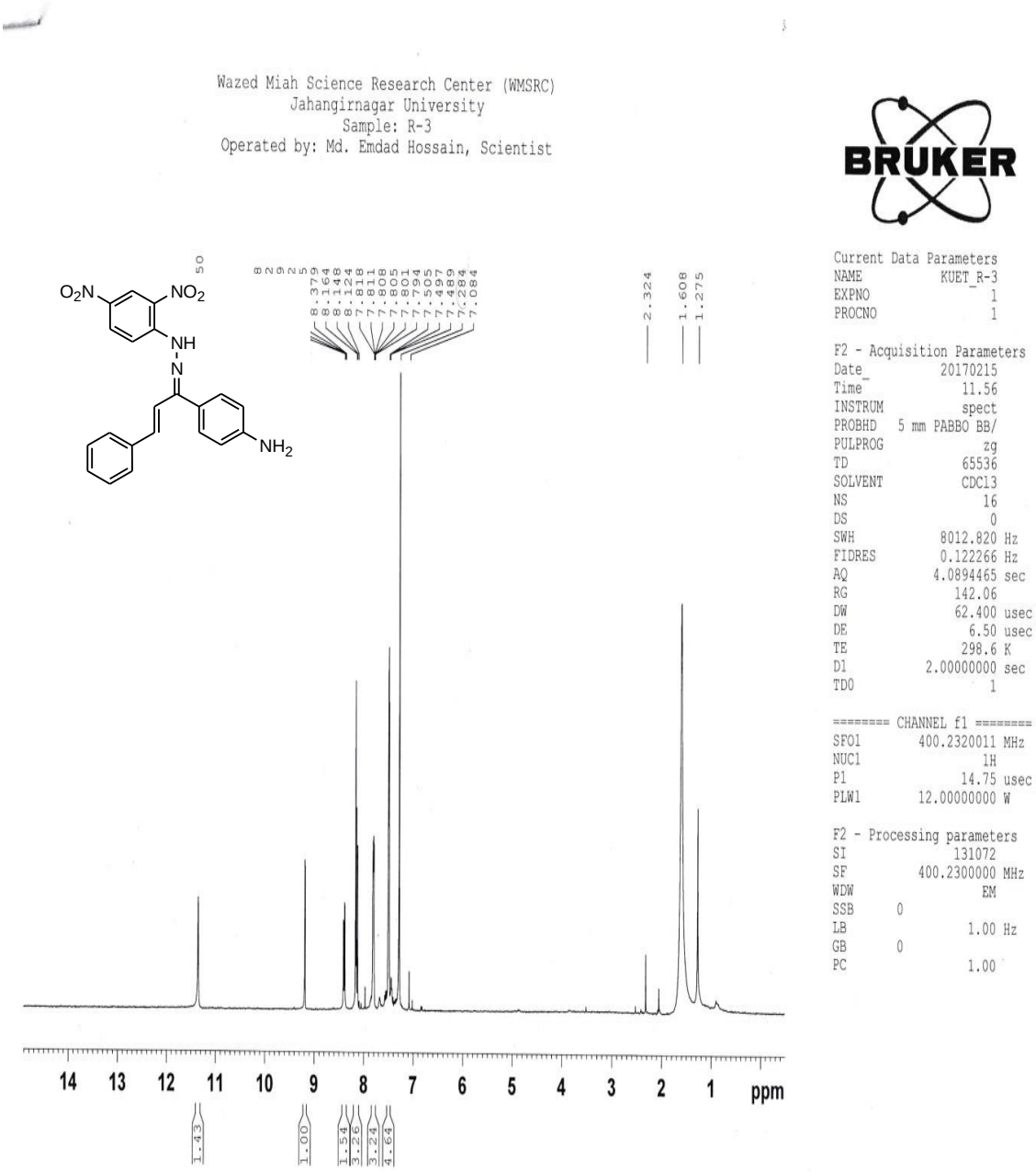


Figure 4.6: NMR spectrum of the compound (R.P-3) in $CDCl_3$

4.4 Antimicrobial Activity

4.4.1 Reading plates and interpreting results for bacteria

The TSA plates culture plates were incubated at 37°C for 24 hours. The zones of inhibition produced by compounds and ciprofloxacin were recorded in mm and compared. The Inhibition zone of some selected active compounds was given below:

Figure 4.7: Antibacterial activity of compounds 7a, 8a, 9a against *Staphylococcus aureus*

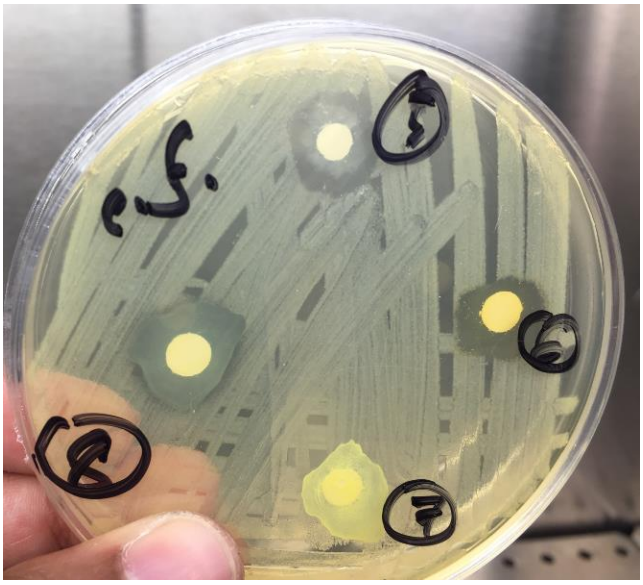


Figure 4.8: Antibacterial activity of compounds 7a, 8a, 9a against *Citobacter freundi*

4.4.2 Reading plates and interpreting results for fungi

The SDA plates culture plates were incubated at 30°C for 24 hours. The zones of inhibition produced by compounds and Michonazole were recorded in mm and compared. The Inhibition zone of some selected active compounds was given below,

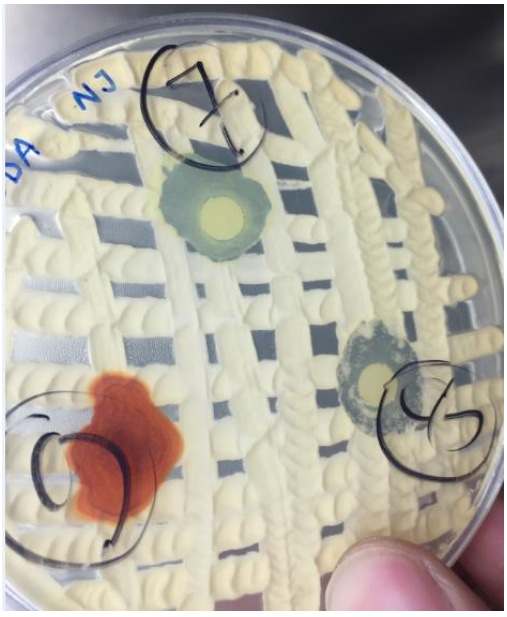


Figure 4.9**:** Antifungal activity of compounds 7a, 8a, 9a against *Tricodarma harzianu*

Table 4.4: Antibacterial and Antifungal activity of the synthesized compounds

Tasted sample	Name of Bacteria						Name of fungi
	Diameter of zone of inhibition in (mm)						
	B. cereus	*S. aureus*	*L. monocytogenes*	*E. coli*	*S. typhimurim*	*C. freundi*	*T. harzianum*
01*	0	8	0	0	0	0	10
02*	6	8	0	0	0	13	8
03*	0	0	0	0	0	0	6
04*	8	14	15	0	0	14	14
05*	13	16	0	12	10	12	20
06*	12	15	0	15	13	12	17
07	0	0	0	0	0	0	11
08	11	16	0	16	11	14	0
09	0	0	0	0	0	24	0
Standard	30	32	40	35	30	35	-

Compound 1= (4-hydroxy phenyl)- 1-phenyl-propenone **Compound 7**=1-(4-Aminophenyl)-3-phenyl-propene

Compound 2= 4-(4-Hydroxy-phenyl)-7,7-dimethyl-2-phenyl-4,6,7,8-tetrahydro-chromen-5-one

Compound 3 = 4-{3-[(1-Methylene-4-nitro-but-2-enyl)-hydrazono]-3--phenyl-propenyl}-phenol

Compound 4 = 1,3-diphenyl-propanone

Compound 5= 2-Etoxy-4,6-diphenyl-5,6-dihydro-4H-pyran-3-carboxylic acid propyl esterand

Compound 6 = N-(2,4-Dinitro-phenyl)-N$^{/}$ -(1,3-diphenyl-allylidene)-hydrazine

Compound 8 = 7-(4-Amino-phenyl)-5-phenyl-1,5-dihydro-pyrano [2,3-d] pyrimidine-2,4-dione

Compound 9=4-{1-[2,4-Dinitro-phenyl)-hydrazono]-3-phenyl-allyl}-phenylamine

*** For comparison with other substituent in our research group.**

The graphical representation of antimicrobial properties of the synthesized compound 7a, 8a and 9a has given below:

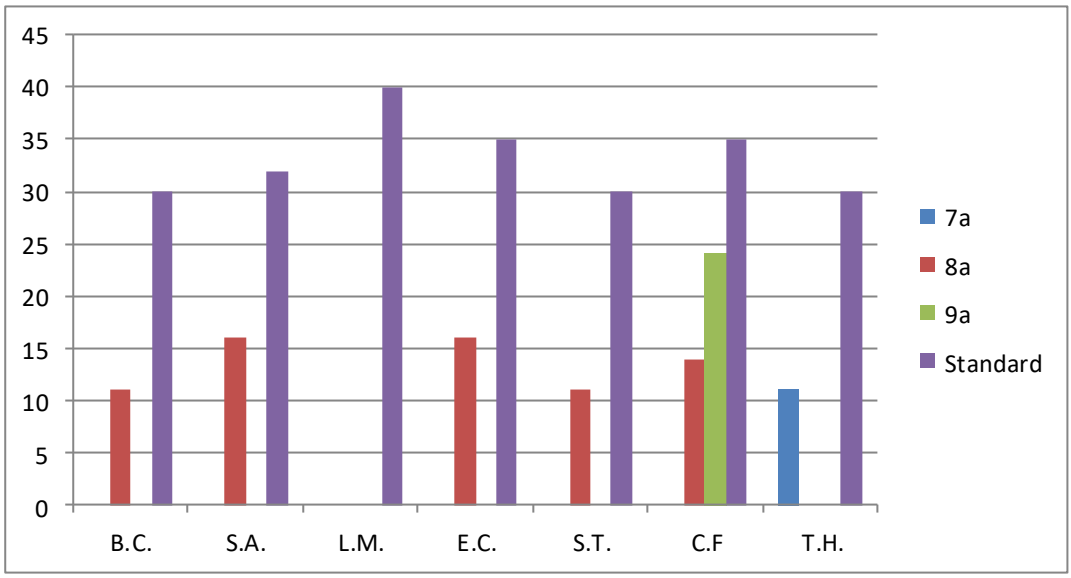


Figure 4.10: Zone of inhibition vs. bacterial and fungal strain of the synthesized compound 7a, 8a, 9a.

Elaboration of bacterial and fungal species on the chart

B.C = *Bacillus cereus*

S.A = *Staphylococcus aureus*

L.M = *Listeria monocytogenes*

E.C = *Escherichia coli*

S.T = *Salmonella typhimurium*

C.F = *Citobacter freundi*

T.H = *Tricodarma harzianum*

4.4.3 Discussion

The synthesized compound of 7a, 8a, 9a were screened for their antibacterial activity against both three Gram positive and three Gram negative bacteria and antifungal activity against *Tricodarma harzianum* by disc diffusion method using Ciprofloxacin and Michonazole as standard and DMSO as the vehicle. Screenings for the all synthesized compound were done

at 300µg/disc. *Ctirobacter freundi* was found to be resistant for most of the compound. The value of zone of inhabition were 14-24 mm. *Bacillus cereus, Staphylococcus aureus* , *Listeria monocytogenes , Salmonella typhimurium, Escherichia coli were* showed the inhabition zone at 11-16 mm which for the compound of 8a. From the data it is observed that Compound 7a exhibits no activity against the tested bacterial strains but showed moderate activity against the tested fungi *Trichoderma harziniaum* having inhibition zone 11 mm compared to the standard used of Michonazole due to the presence of phenyl amino group. Compound 8a exhibits broad spectrum activity against both gram negative and gram positive strains tested except *Listeria monocytogenes*. Thus due to the presence of reactive amino group and unsaturated ketone function containing the heterocyclic rings in the synthesized compound 8a, it enhance the antimicrobial activity. Also the compound 9a showed promising antibacterial activity having inhibition zone of 24 mm against *Ctirobacteria freundi* due to their presence of nitro and hydrazine group. Compound 8a, {7-(4-Amino-phenyl)-5-phenyl-1,5-dihydro-pyrano[2,3-d]pyrimidine-2,4-Dione} showed good activity which was synthesized from compound 8a . Compound 9c; {4-{1-[(2,4-Dinitro-phenyl)-hydrazono]-3-phenyl-allyl}-phenylamine} showed enhanced antibacterial activities.

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