Synthesis and Antimicrobial Activities of Chromene Derivatives

by

Md. Tarikul Islam

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

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Khulna 9203, Bangladesh

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DADICATED

TO MY

FAMILY

Declaration

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Associate Professor
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Dhaka University
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Md. Tarikul Islam

Signature of Candidate

Abstract

The design and synthesis of chalcone and chromene derivatives and their antibacterial activities were studied in this research. Conveniently, all products were synthesized from commercially available acetophenone, 4-hydroxybenzaldehyde and dimedone. Different synthetic strategies were used to obtain an easily accessible chromene scaffold with appropriate handles. At first, this research work was involved in an efficient procedure for the attachment of 4-hydroxybenzaldehyde and acetophenone in the presence of 10% NaOH to produce 3-(4-Hydroxy-phenyl)-1-phenyl-propenone. 4-{3-[(2,4-Dinitro-phenyl)-hydrazono]-3-phenyl-propenyl}-phenol phenylamine has been synthesized by the reaction of 2,4 dinitro phenyl hydrazine with chalcone. Reaction of dimedone with chalcone under refluxing condensation using dry benzene and zinc chloride as a catalyst to give the corresponding chromene derivative 4-(4-Hydroxy-phenyl)-7,7-dimethyl-2-phenyl-4,6,7,8-tetrahydro-chromen-5-one. This reaction also carried out by Microwave Irradiation and use ethanol as a solvent, without any catalyst. It was found that the preparation time for the compounds was reduced from 72 hours to 5-10 minutes during microwave irradiation. The structures of the all synthesized compounds were characterized by their IR, ^{1}H NMR spectral data. The antibacterial and antifungal activity of the compounds was also investigated. The antimicrobial activity of the compounds at concentration 300 µg/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,** 3-(4-Hydroxy-phenyl)-1-phenyl-propenone exhibits no activity against the tested bacterial strains *Bacillus cereus*, *Listeria monocytogenes, Escherichia coli, Salmonella typhimurium, Citrobacter freundii,* but showed moderate activity against the tested bacteria *Staphylococcus aureus* having inhibition zone 8 mm and also show antifungal activity aganist *Trichoderma harzianum* inhibition zone 10mm. Compound, **II,** 4-{3-[(2,4-Dinitro-phenyl)-hydrazono]-3-phenyl-propenyl}-phenol exhibits no activity against gram negative, gram positive strains but showed antifungal activity against

Trichoderma harzianum inhibition zone 6 mm. Compound, **III**, 4-(4-Hydroxy-phenyl)-7,7-dimethyl-2-phenyl-4,6,7,8-tetrahydro-chromen-5-one showed activity against Gram positive, Gram negative except *Listeria monocytogenes, Escherichia coli, Salmonella typhimurium*. This compound, **III** also showed antifungal activity against *Trichoderma harzianum* inhibition zone 8 mm.

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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
δ	Chemical shift
TMS	Tetra methyl Silane
D_6	Deuterated Dimethyl sulfoxide

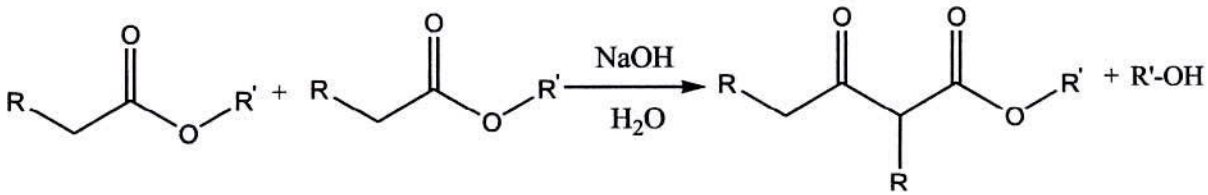
CHAPTER I

INTRODUCTION

Introduction

1.1. General:

The Claisen condensation is a carbon-carbon bond forming reaction that occurs between two esters or one ester and another carbonyl compound in the presence of a strong base, resulting in a β-keto ester or a β-diketone [1]. It is named after Rainer Ludwig Claisen, who first published his work on the reaction in 1887 [2-4].



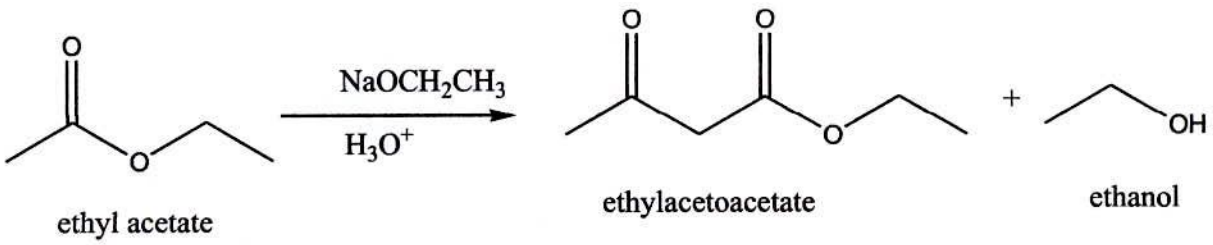
Scheme. 1.1

1.1.1. Requirements

At least one of the reagents must be enolizable (have a α-proton and be able to undergo deprotonation to form the enolate anion). There are a number of different combinations of enolizable and nonenolizable carbonyl compounds that form a few different types of Claisen condensations. The base used must not interfere with the reaction by undergoing nucleophilic substitution or addition with a carbonyl carbon. For this reason, the conjugate sodium alkoxide base of the alcohol formed (e.g. sodium ethoxide if ethanol is formed) is often used, since the alkoxide is regenerated. In mixed Claisen condensations, a non-nucleophilic base such as lithium diisopropylamide, or LDA, may be used, since only one compound is enolizable. LDA cannot be used in the classic Claisen or Dieckmann condensations, since virtually all ester will be converted to ester enolate and condensation will not occur. The alkoxy portion of the ester must be a

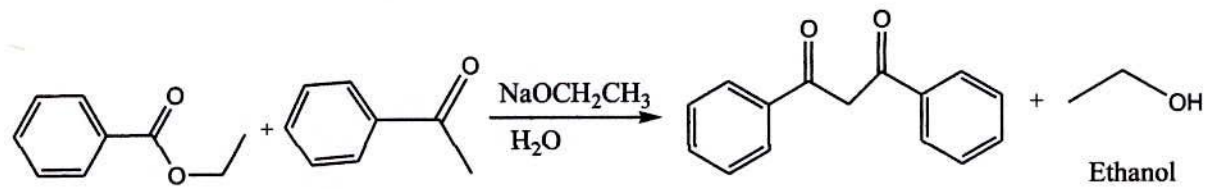
good leaving group. Methyl and ethyl esters, which yield the methoxy and ethoxy leaving groups, respectively, are usually used.

1.1.2. Types of Claisen condensation



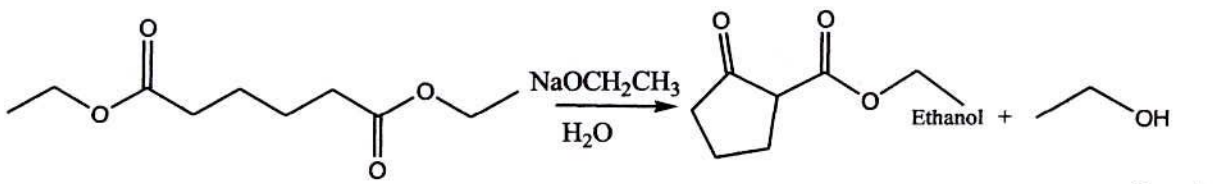
ethyl acetate ethylacetoacetate ethanol

The classic Classion condensation, where only one enolizable ester is use



ethyl benzoate acetophenone 1,3-diphenylpropane-1,3-dione Ethanol

The mixed (or crossed) Claisen condensation, where an enolate ester and a nonenolizable ester are use.



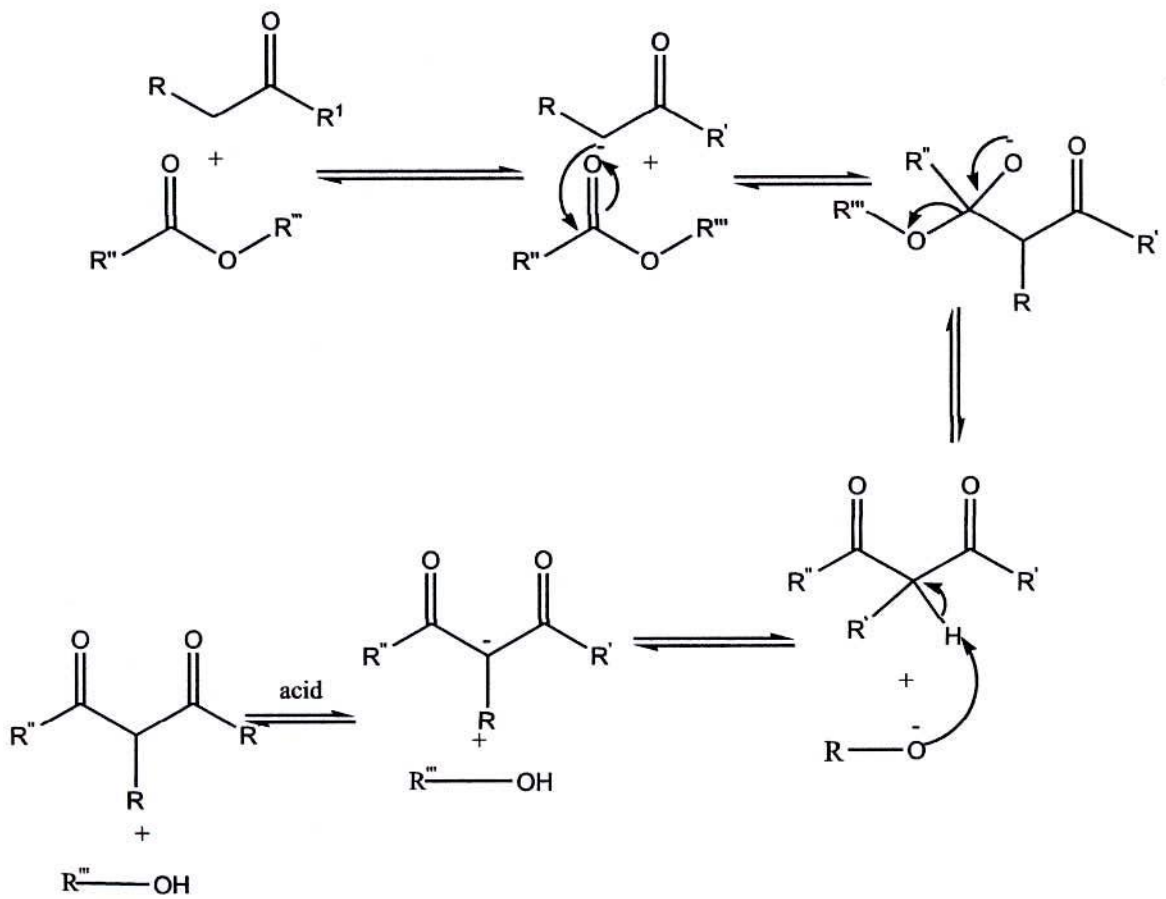
dethyl adipate ethyl-2-oxocyclopentanecarboxylate ethanol

Scheme 1.2

The Dieckmann condensation , where a molecule of two ester groups react intramolecularly, forming a cyclic β-keto ester. In this case the ring formed must not be straight, usually a 5- or 6- member chain or ring.

1.1.3. Mechanism

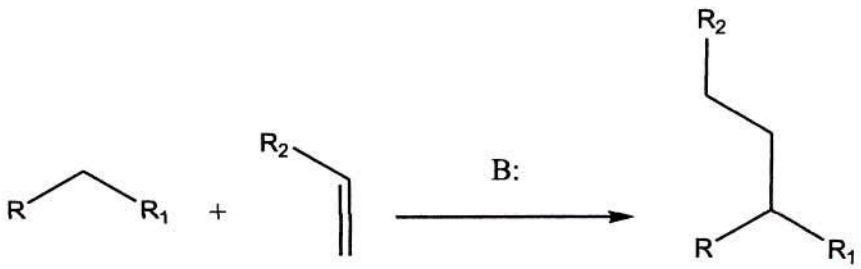
In the first step of the mechanism, an α-proton is removed by a strong base, resulting in the formation an enolate anion, which is made relatively stable by the delocalization of electrons. Next, the carbonyl carbon of the (other) ester is nucleophilically attacked by the enolate anion. The alkoxy group is then eliminated resulting in regeneration of the alkoxide), and the alkoxide removes the newly-formed doubly α-proton to form a new, highly resonance-stabilized enolate anion. Aqueous acid (e.g. sulfuric acid or phosphoric acid) is added in the final step to neutralize the enolate and any base still present. The newly-formed β-keto ester or β-diketone is then isolated. Note that the reaction requires a stoichiometric amount of base as the removal of the doubly α-proton thermodynamically drives the otherwise endergonic reaction. That is, Claisen condensation does not work with substrates having only one α-hydrogen because of the driving force effect of deprotonation of the β-keto ester in the last step [5].



Scheme. 1.3

1.2 Michel Reaction

The Michael reaction or Michael addition is the nucleophilic addition of a carbanion or another nucleophile [6-7] to an α,β-unsaturated carbonyl compound. It belongs to the larger class of conjugate additions. This is one of the most useful methods for the mild formation of C–C bonds. [8] Many asymmetric variants exist.



Scheme. 1.4

In this scheme the R and R' substituents on the nucleophile (a Michael donor) are electron-withdrawing groups such as acyl and cyano making the methylene hydrogen acidic forming the carbanion on reaction with base B:. The substituent on the activated alkene, also called a Michael acceptor, is usually a ketone making it an enone, but it can also be a nitro group [9-10].

Arthur Michael in Germany, in 1887 first built up important synthetic methods for the nucleophilic addition to carbon- carbon double bond in conjugation with electron withdrawing groups such as >CO, -COOH, -CN, $-NO_2$, $-SO_2R$, -COOR, $CONH_2$ .Michael reaction has wide application in synthetic organic chemistry. This reaction is commonly use for the alkylation of active methelene compounds. In Michael addition an extention of condensation of aldole type occur [11].

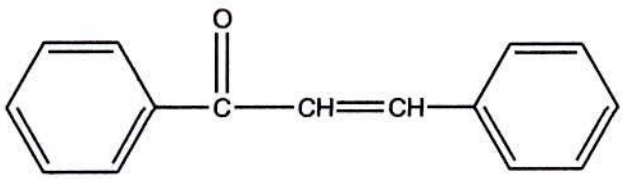
Although a carbon- carbon double bond gives an electrophilic addition. The presence of electro withdrawing groups in conjugation with double bond not only lowers its reactivity toward electron seeking reagents but at the same time activeate towards reagents that are electron rich.

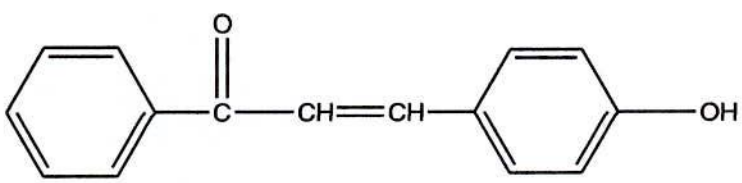
1.2.1 Michael Acceptor

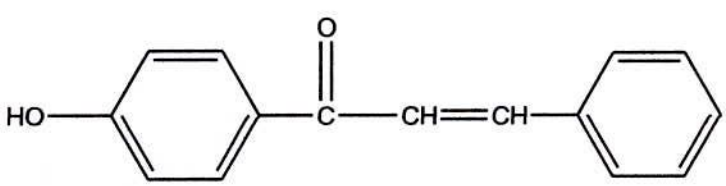
The acceptor compounds are usually α,β-unsaturated esters, aldehydes, ketones and nitriles compounds in which the carbon-carbon double bond and the carbon- oxygen double and are separated by just one carbon-carbon single bond i.e. the double bonds are conjugated, are called α,β-unsaturated carbonyl compounds. The structural moiety is as follows:

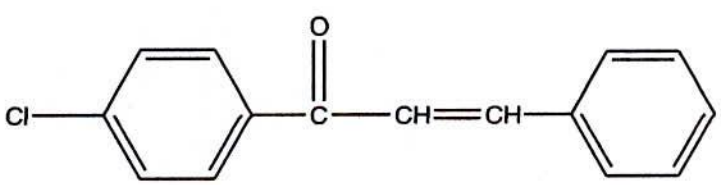
$-C_{\beta}=C_{\alpha}-C=O$

Some α, β- Unsaturated carbonyl componds are cited beloe:



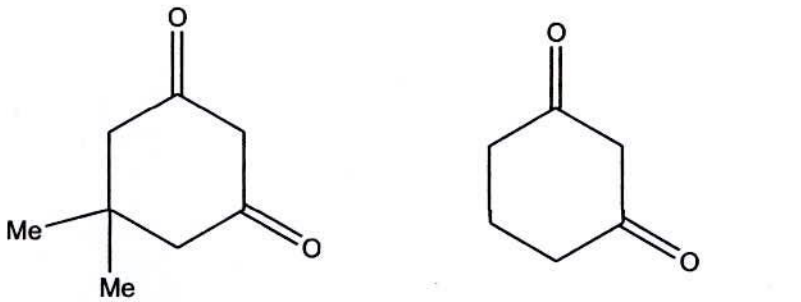






1.2.2 Donor compound

Compound that contain a $-CH_2$ group and –CH group flanked by two electron with drowning groups (_CN, $-OOC_2H_5$,>CO) Which can increase the activity of hydrogen and stabilized the conjugate base and called active hydrogen compounds. Some active hydrogen compound are below [[12].



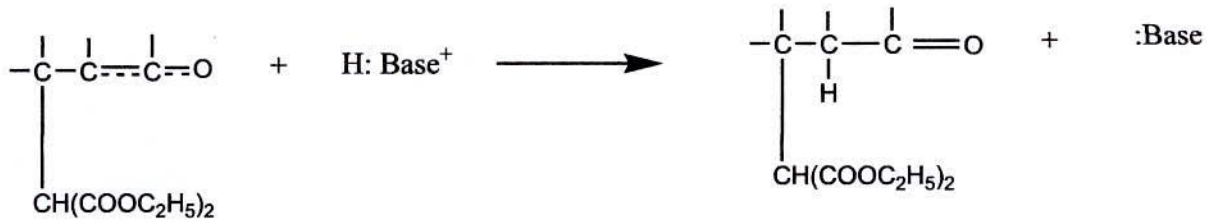
Dimedone 1,3-cydohexanedione

Fig 1.1: Donar compound

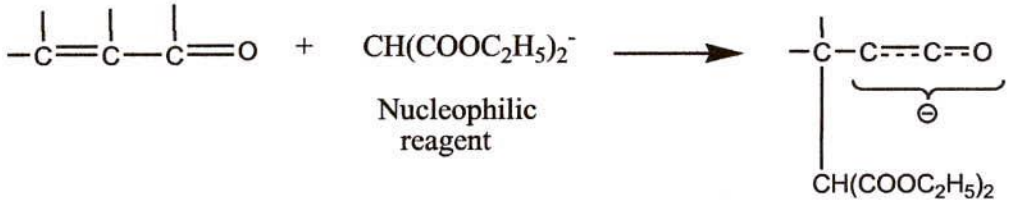
1.2.3 Mechanism of Michael reaction [13]

First step:

Second step:



Third step:



Scheme. 1.5

1.3. Chalcone

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 [14]. Chalcones are α, β-unsaturated ketones consisting of two aromatic rings (ring A and B) having diverse array of substituents. Rings are interconnected by a highly electrophilic three carbon α,β-unsaturated carbonyl system that assumes linear or nearly planar structure [15-17]. They contain the ketoethylenic group (–CO–CH=CH-) [18].

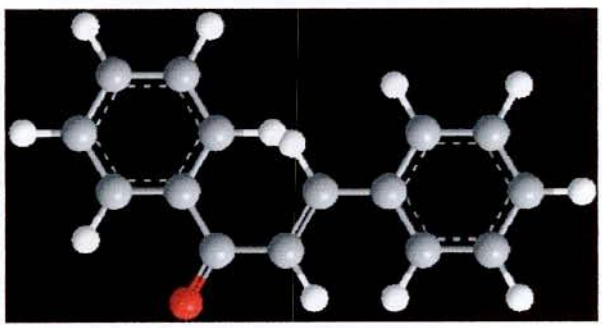
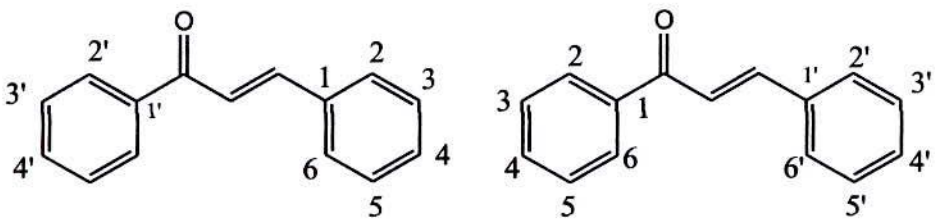


Fig 1.2: 3D structure of Chalcone

Different methods of nomenclatures for chalcone were suggested at different times. The pattern (a) has been adopted by "chemical abstracts" published by American chemical society. The British chemical abstract and journal of chemical society have followed pattern (b) [19].



(a) (b)

Fig 1. 3: Chalcone

Chalcones or 1, 3-diaryl-2-propen-1-ones, belong to the flavonoid family. Chemically they consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α, β -unsaturated carbonyl system. Chalcones are the precursors in the biosynthesis of anthocyanins and flavones. Chalcones and substituted chalcones can be synthesized in laboratory by Claisen-Schmidt condensation of acetophenone or substituted acetophenones with aldehydes. The first condensation was reported by Kestanecki and he gave the name "Chalcones" [20].

An interesting feature of chalcones is that they serve as starting materials for the synthesis of various heterocyclic compounds such as pyrimidines, pyrazolines, flavones, flavonols, flavanones, aurones and benzoylcoumarones as well as certain compounds like deoxybenzoins and hydantions which are of some therapeutic importance.
The chalcones are coloured compounds because of the presence of the chromophore (-COCH=CH-). In fact, the pharmacological properties of chalcones are due to the presence of both α, β- unsaturation.

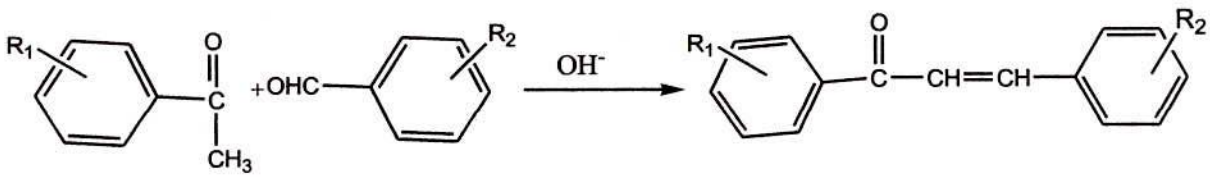
Chalcones are natural compounds that are largely distributed in plants, fruits, and vegetables. They belong to the flavonoid group of molecules and some of them exhibit numerous biological activities. They are precursors in flavonoid biosynthesis. The enzymatic cyclization of the 6'-hydroxychalcones leads to the formation of flavanones and subsequently to a large number of flavonoid groups including flavones, flavonols, dihydroflavonols, aurones and isoflavones [21].
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). 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 [22].

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 [23].

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

Variety of methods are available for the synthesis of chalcones . The most convenient method is the one that involves the Claisen - Schmidt condensation of an equimolar quantities of a substituted acetophenone with substituted enzaldehyde in the presence of an aqueous alcoholic alkali [24]. In the Claisen- Schmidt reaction, the concentration of alkali used usually ranges between 10 to 60 %. The reaction is carried out at room temperature for 24 hours.



Scheme. 1.6

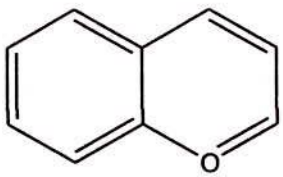
Chalcone bears a very good synthon so that variety of novel heterocyclic with good pharmaceutical profile can be designed. In recent years there has been a lot of work done in preparation of chalcones containing heterocyclic. Chalcones have been used as intermediates for the preparation of compounds 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, antimicrobial, antileishmanial, anti-inflammatory, antitumour and antibacterial activity. 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 [25].

1.4. Chromene:

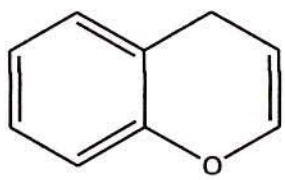
Benzopyran is an polycyclic organic compound that results from the fusion of a benzene ring to a heterocyclic pyran ring. According to current IUPAC nomenclature, the name chromene used in previous recommendations is retained; however, systematic 'benzo' names, for example 2H-1-benzopyran, are preferred IUPAC names for chromene, isochromene, chromane, isochromane, and their chalcogen analogues [26]. There are two isomers of benzopyran that vary by the orientation of the fusion of the two rings compared to the oxygen, resulting in 1-benzopyran (chromene) and 2-benzopyran (isochromene - the number denotes where the oxygen atom is located by standard naphthalene-like nomenclature.

The radical form of benzopyran is paramagnetic. The unpaired electron is delocalized over the whole benzopyran molecule, rendering it less reactive than one would expect otherwise, a similar example is the cyclopentadienyl radical. Commonly, benzopyran is encountered in the reduced state, in which it is partially saturated with one hydrogen atom, introducing a tetrahedral CH_2 group in the pyran ring. Therefore, there are many structural isomers owing to the multiple possible positions of the oxygen atom and the tetrahedral carbon atom:



2H-chromene

2H-1-benzopyran



4H-chromene

4H-1- benzopyran

Structural isomers of iso chromene

1H -isochromene
1H-2-benzopyran

3H-isochromene
3H-2-benzopyran

Fig 1.4: Chromene and iso chromene

The chromone ring system, 1-benzopyran-4-one (Figure 1.6), is the core fragment in several flavonoids, such as flavones, flavonols and isoflavones [27]. The word chromone can be derived from the Greek word chroma, meaning "color", which indicates that many chromone derivatives exhibit a broad variation of colors.

The rigid bicyclic chromone fragment has been classified as a privileged structure in drug discovery (i.e. a molecular framework able to provide ligands for diverse receptors), due to its use in a wide variety of pharmacologically active compounds such as anticancer, 5 anti-HIV, antibacterial and anti-inflammatory agents [28-29]. Several chromone derivatives have also been reported to act as kinase inhibitors, to bind to benzodiazepine receptors and as efficient agents in the treatment of cystic fibrosis [30].

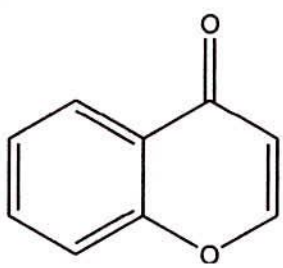


Fig 1.5: General structur of chromone

The collective term `chromones' is used for chemical compounds that contain the structure (1.6) fig, some of which are used in clinical medicine. The chromone structure is found within the chemical structure of the avonoids, a group of naturally occurring substances that are of current interest because of their antioxidant activity. Khellin was first prepared

in impure form by Mustapha in 1892 [31]. Pharmacologically it was studied by Samaan in 1932 who showed that it relaxes all visceral smooth muscles by direct action on the muscle fibres [32]. The main active ingredient was isolated in its pure form by Ernst Spath in Vienna in 1934 [33]. He also established the structure analytically. Khellin was first synthesized by Dr (later Professor) G. R. Ramage in conjunction with R. A. Baxter and N. R. Timson at the British Schering Research Institute at Alderley Edge in Cheshire, UK in 1947 [34].

Although there are a large number of chromone derivatives known for their pharmacological properties there are only a few examples that have been or that are used as therapeutic agents today. Khellin (Figure 1.6), extracted from the seeds of the plant Ammi visnaga, was the first chromone in clinical practice and it has been used for centuries in the Mediterranean area as a diuretic to relieve renal colic [35].

The most common synthetic routes to the chromone structure occur via a chalcone intermediate or via the Baker-Venkataraman rearrangement (Scheme 1.6) [36]. The chalcone pathway implicates the base-catalyzed aldol condensation of 2′-hydroxyacetophenones with aromatic or conjugated aldehydes. The resulting chalcone can then be cyclized to a flavone (e.g. in the presence of iodine) or to the corresponding 3-hydroxyflavone, using alkaline hydrogen peroxide solution, via the Algar-Flynn-Oyamada (AFO) reaction [37].

The Baker-Venkataraman approach involves rearrangement of O-acetylated 2′-hydroxyacetophenones to ortho-hydroxy 1,3-diketones via enolate formation followed by a base-promoted acyl transfer. The chromone structure can then be obtained via acid catalyzed cyclization. Several alternative routes to obtain chromones and flavones have been reported over the recent years, such as the cyclization of alkynyl-ketones (either base promoted or using iodine monochloride) or palladiummediated cyclocarbonylation of ortho-iodophenols with terminal acetylenes in the presence of carbon monoxide [38].

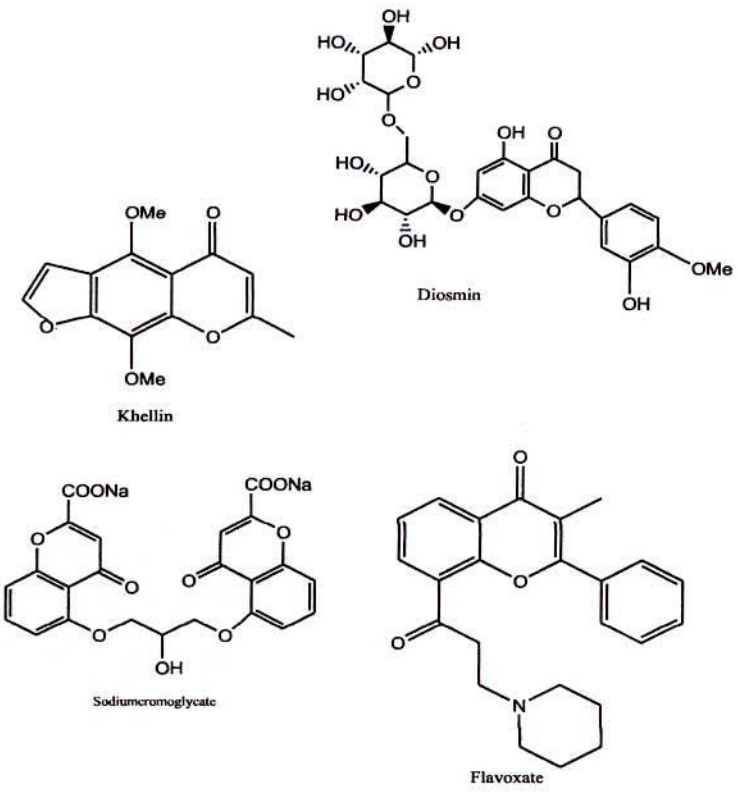
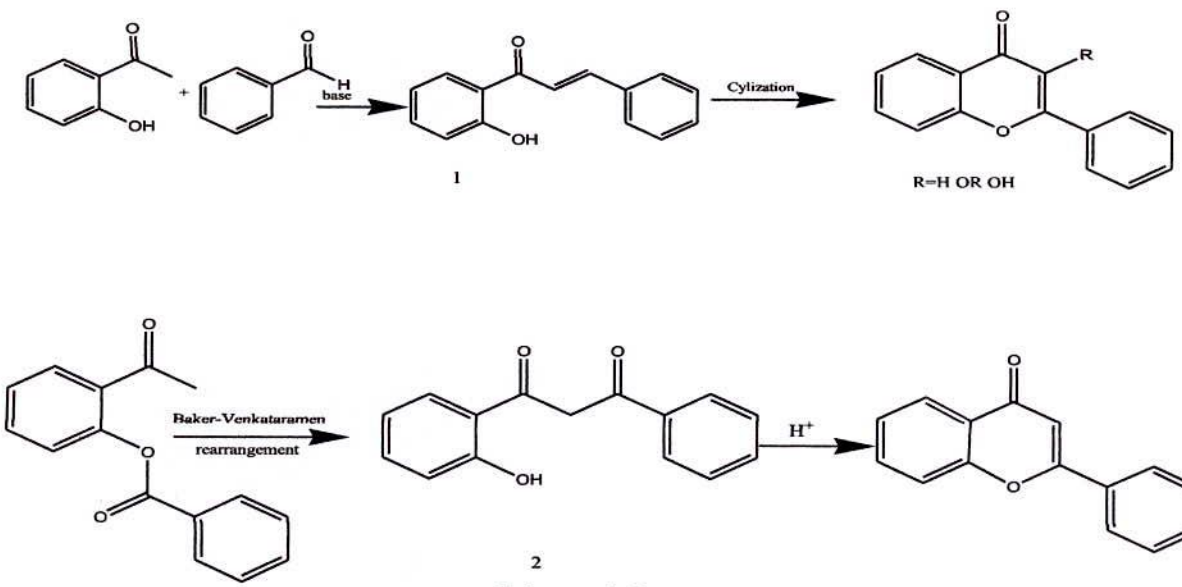


Fig 1.6: Examples of chromone-based compounds

Another common synthetic route to the chromenes derivative occurs via a chalcone intermediate by Michael addition reaction. This method $ZnCl_2$ as a catalyst and dimedone and chalcon are reactant [39].



Scheme. 1.6

This thesis includes the design and synthesis of chalcones and chromene derivatives by Claisen condensation and Michael addition reaction respectively. The specific objectives of the thesis were:

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

CHAPTER II

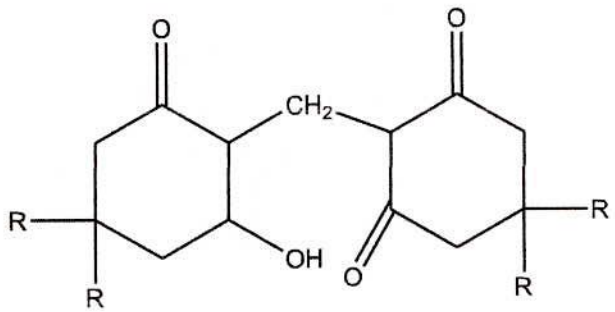
LITERATURE REVIEW

CHAPTER II

Literature Review

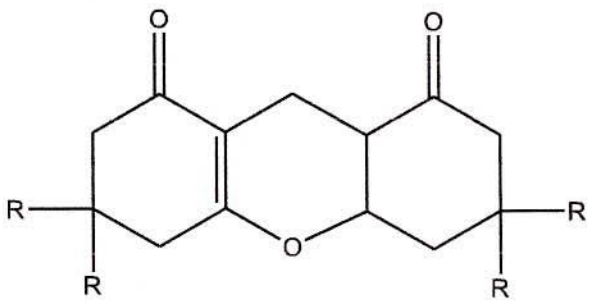
2.1 General:

The well-known reaction of dimedone (5, 5-dimethyl-1, 3-cyclohexanedione (1) [R=Me] in saturated aqueous solution or in 10% alcoholic solution gave crystalline derivative (1) with aldehides but not with ketones .



1

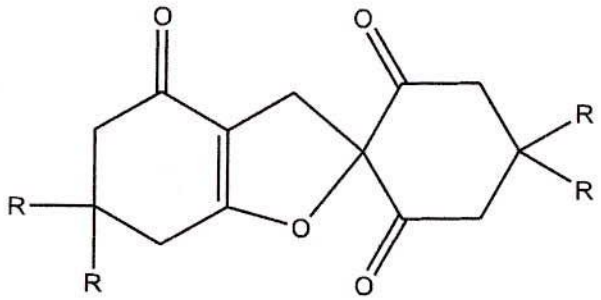
The crystal derivative, alkylidine dimedone (1) upon boiling with glacial acetic, acetic anhydride, hydrochloric acid frequently loses water and gave a substituted octahydroxanthene or the anhydride (2) [40].



2

The reaction of dimedone with formaldehyde has been studied by a number of workers. Fredith greenberge studied this reaction by the treatment of disodium salt of dimedone derivative of formaldehyde with iodine and obtain product (3). The structure was

supported by NMR and IR spectral datd. This compound (3) when subjected to rodium hydroxide treatment in aqueous dioxane followed by acidification gave a compound whose.



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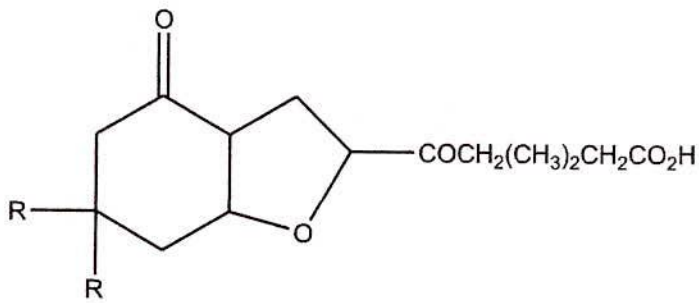
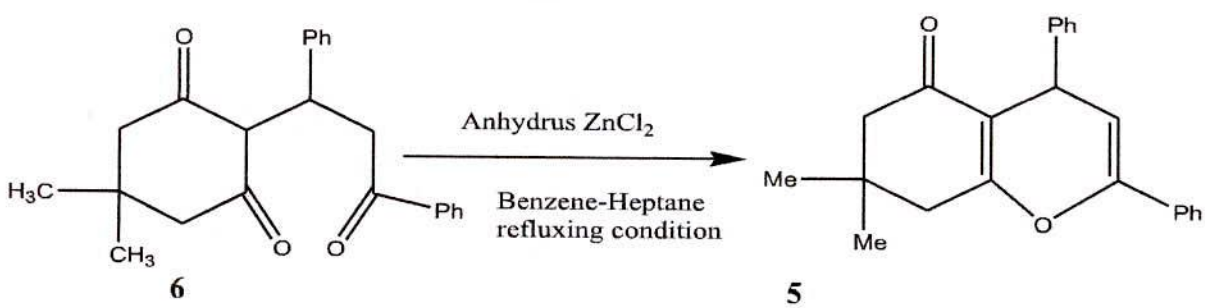


4

Spectral values coincide with those of structure (4). The dominant factor leading to the formation of (3) may be due to greater stability of dimedone in enol rather than the keto form [41].

Ahmad abd co-workers studied the Zncl2 catalyzed reaction of dimedone and chalcone and reported the formation of (5). The same product (5) was also obtained from (6) which was the 1:1 adduct of dimedone and chalcon [42].

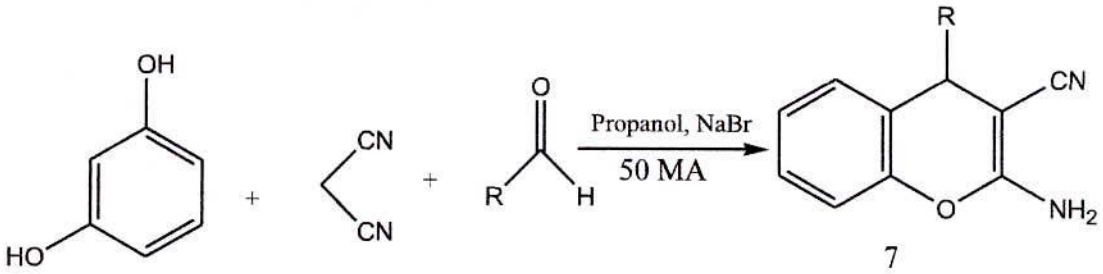


6 **5**

Scheme. 2.1

2.2 Rivew of 4-H-Chromene

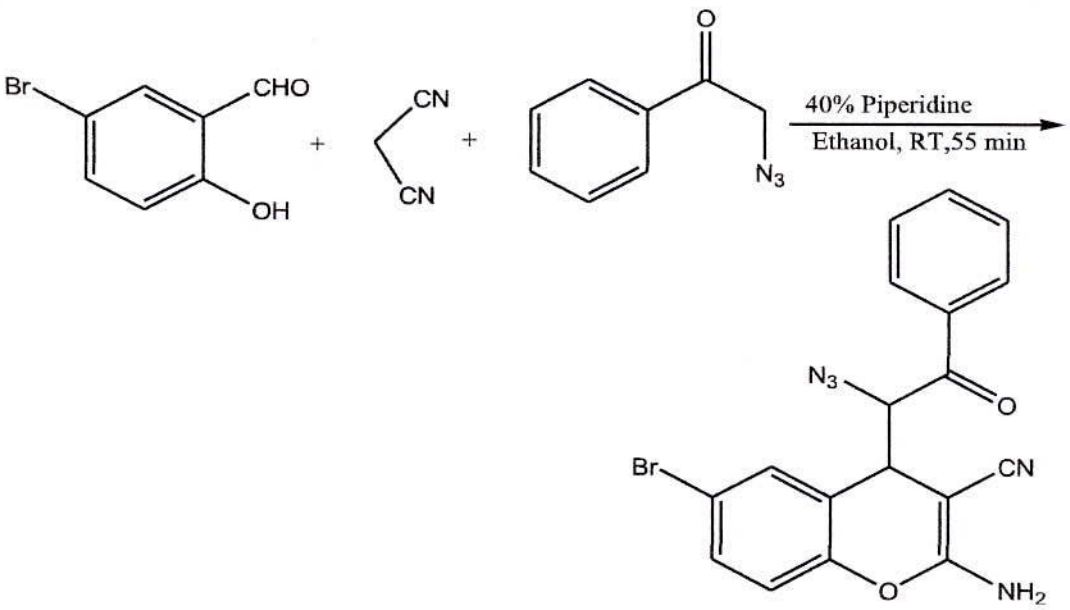
S.Makaram and co-workers developed electro chemically induced multi-component condensation of resorcinol, malononitrile, and various aldehydes in propanol in an undivided cell in the presence of NaBr as an electrolyte results in the formation of 2-amino-4H-chromenes (7) in good yields and short reaction time [43].



7

Scheme. 2.2

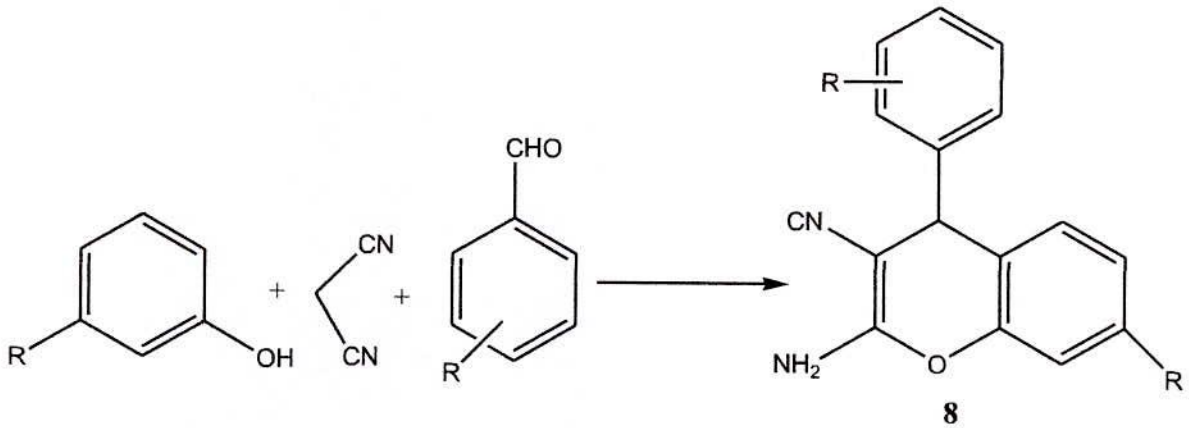
T. H. Babu et.al.reported an efficient and simple synthesisof highly functionalized azidochromene derivatives. This achieved by Michael addition of α-azido ketones on iminocoumarin derivatives obtained from salicylaldehydes and malononitrile. Synthesized azidochromenes were successfully transformed into triazolylchromenes by the [3+2] cycloaddition reaction [44].



7a

Scheme. 2.3

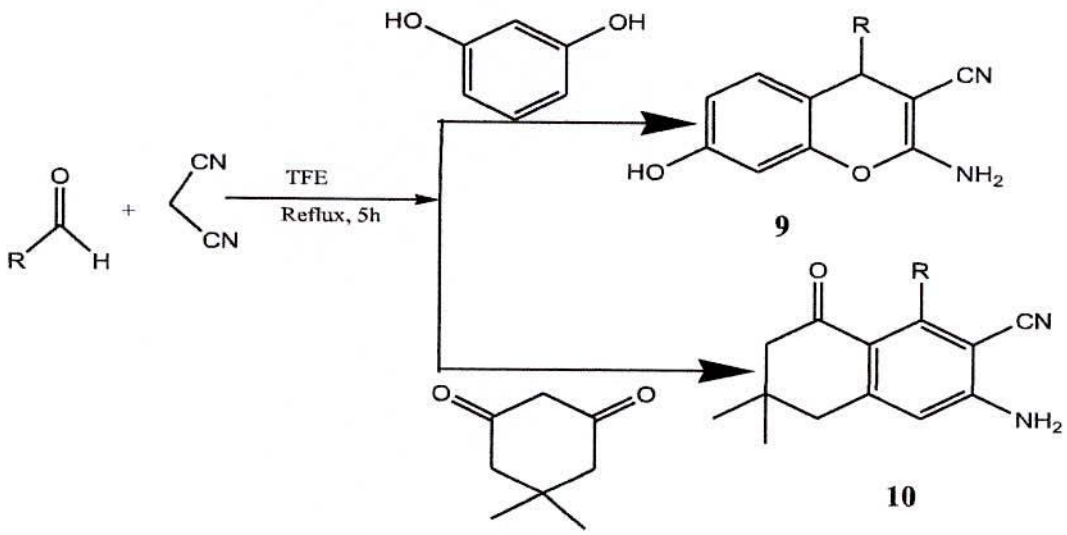
S.R. Kolla et.al. prepared variety of novel 2-amino-5- hydroxy-4H-chromene derivatives (8) with various substituents on the 4Hchromenering were efficiently synthesized by onepot reactions of substituted resorcinols and various2- benzylidene malononitriles in the presence of calcium hydroxide in methanol at room temperature. This simple method provided 2-amino-5-hydroxy-4H-chromenes (8) with high yields under mild reaction conditions [45].



8

Scheme. 2.4

S.Khaksaret.al .synthesized highly efficient one-pot three component regioselective synthesis of 2-amino-3-cyano-4Hchromene (9) and tetrahydrobenzo pyran derivatives (10) has been developed with good yield by annulation of aldehydes, malononitrile, and resorcinol or dimedone under refluxconditions in 2,2,2-trifluoroethanol without the use of a catalyst or any other additive [46].



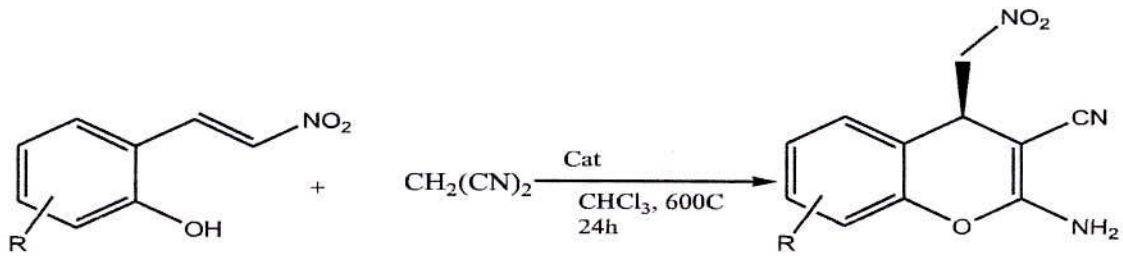
9

10

Scheme. 2.5

Recently, Yu. Gao. and coworkers developed organocatalytic enantioselective tandem Michael addition–cyclization of malononitrile to nitroalkenes for the direct synthesis of chiral 2-amino-4H-chromene-3-carbonitrile derivatives was investigated. Good yields and enantioselectivities (up to 91%) were achieved. This organocatalytic asymmetric tandem.

Michael addition–cyclization provides an efficient route toward the synthesis of optically active functionalized chromenes [47].

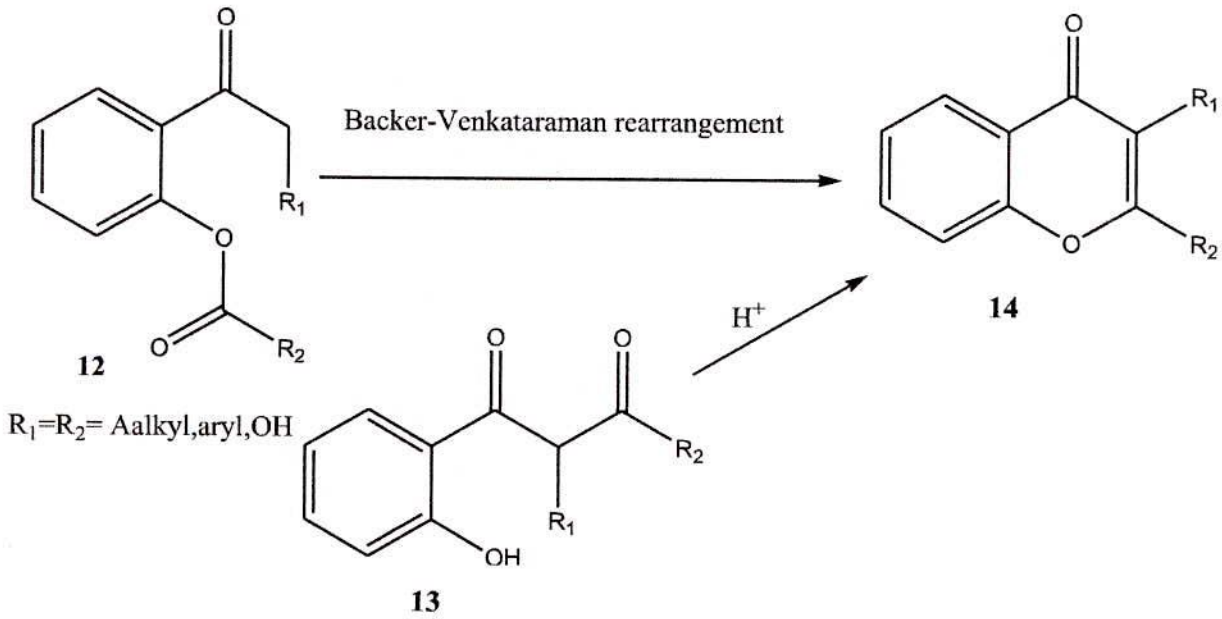


Cat-subsituted thio urea **11**

Scheme. 2.6

2.3 Rivew of Chromene

Chromenes could be synthesized under either acidic or basic conditions. The classical, 3-disubstituted benzopyranone 14 synthesis utilized acidic conditions (Scheme 2.7) and was by far themost common method [48]. It proceeded through an intramolecular condensation of molecules such as 13, which were usually obtained through a Baker–Venkataraman [49] rearrangement of compound 12, or via a Claisen ester condensation (Scheme 2.7). Most synthesis required harsh acidic conditions as the final step. On the other hand, synthesis utilizing basic conditions typically consisted of piperidine inrefluxing pyridine for several hours to affect ring closure. This was far less common [50]. Recently, microwave heating has also been used to affect ring cyclization [51].



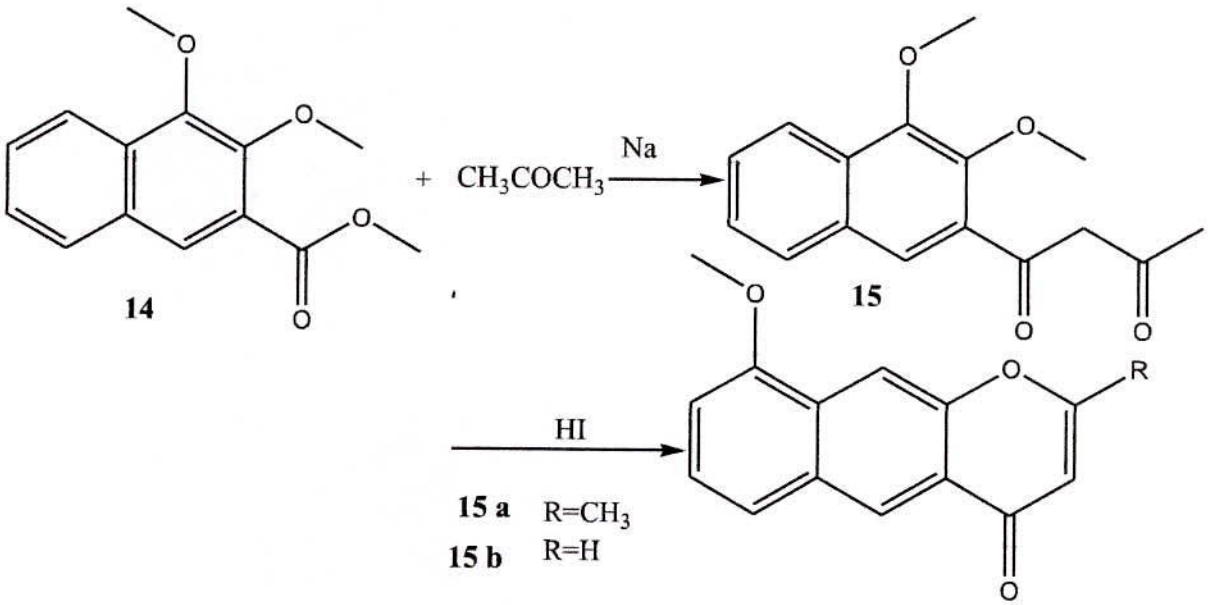
Scheme. 2.7

2.3.1 Acid as catalyst in chromone ring closure:

Acid comprised a major catalyst in chromone ring closure, and many acids can be used includinghydriodic acid, polyphosphoric acid (PPA), acetic acid, methanesulfonylchloride, hydrochloric acid,para toluene sulfonic acid (PTS), triflic anhydride, phosphorus oxychloride, perchloric acid, and sulfuric acid.

2.3.2 Hydriodic acid as a catalyst

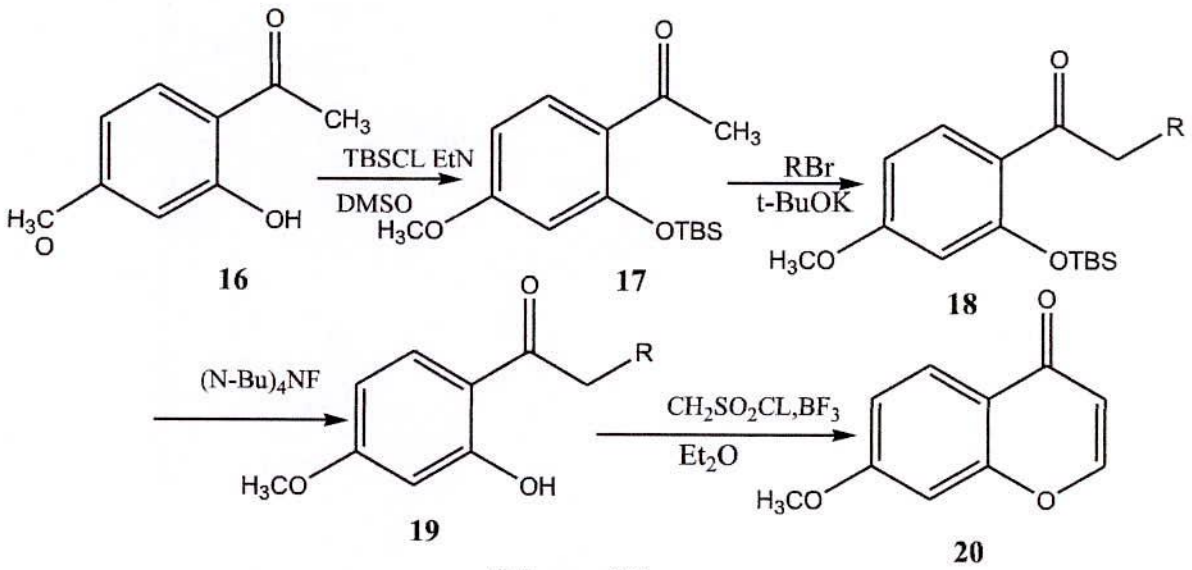
It has been reported that synthesis of a mixture of 2-methyl-8-hydroxy-6,7-benzochromone (15b) and 2-methyl-8-methoxy-6,7-benzochromone (15a) using hydriodic acid as a catalyst in the ring closure [52].



Scheme. 2.8

2.3.3 Methane sulfonyl chloride as a catalyst

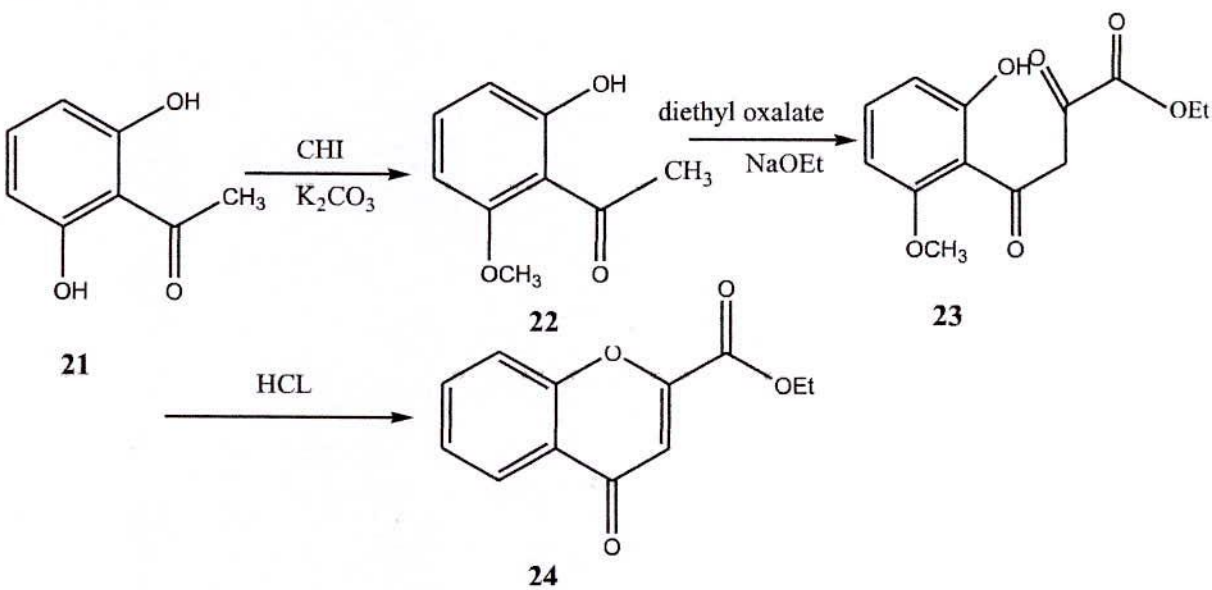
In 2001, Ismail and Abd El Aziem reported the synthesis of the new 3-substituted-7-methoxy-4H-1- benzopyran-4-ones (20) starting from 2-hydroxy-4-methoxyacetophenone (16) according to Scheme 2.9 the key step in this synthesis involved alkylation of2-(t-butyldimethylsilyloxy)-4-methoxyacetophenone (17) with alkyl halide using potassium tertiary butoxide was prepared from 16 by using t-butyldimethyl silylchloride. The o-silyl protected alkylacetophenone derivatives (18) were, therefore, treated with tetra-n-butylammonium fluoride to produce the corresponding 2'-alkyl-2-hydroxy-4-methoxyacetophenone (19) was synthesized in good yield. Cyclization of the alkyl derivatives 19 wasachieved via methane sulfonyl chloride using boron trifluoride diethyl ether at 0°C to give the desired 3-substituted-7-methoxy-4H-1-benzopyran-4ones. This reaction conditions was relatively mild, and the reaction yield was also relatively high [53].



Scheme. 2.9

2.3.4 Hydrochloric acid as a catalyst

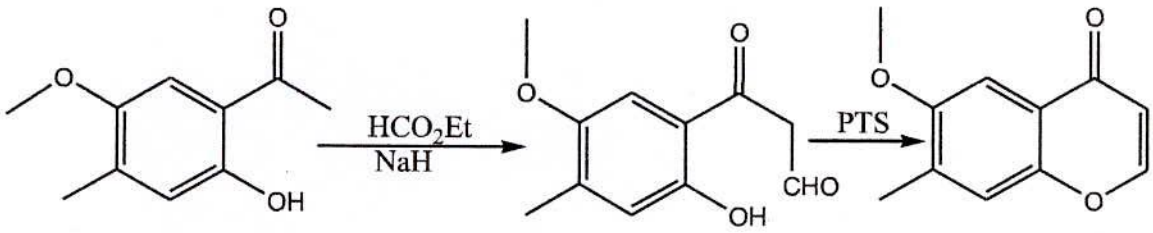
In 2003, Boumendjel and coworkers obtained chromone 24 in three steps starting from 2,6-dihydroxyacetophenone (Scheme 2.10); they used concentrated hydrochloric acid as a catalyst in the ring closure [54].



Scheme. 2.10

2.3.5 Para toluene sulfonic acid as a catalyst

In 2004, Sabui and Venkateswaran synthesized the 6-methoxy-7-methyl chromone 27 using paratoluene sulfonic acid (PTS) as a catalyst in the ring closure (Scheme 2.11). This catalyst was especially suitable in the phenolic hydroxyl and aldehyde condensation cyclization [55].

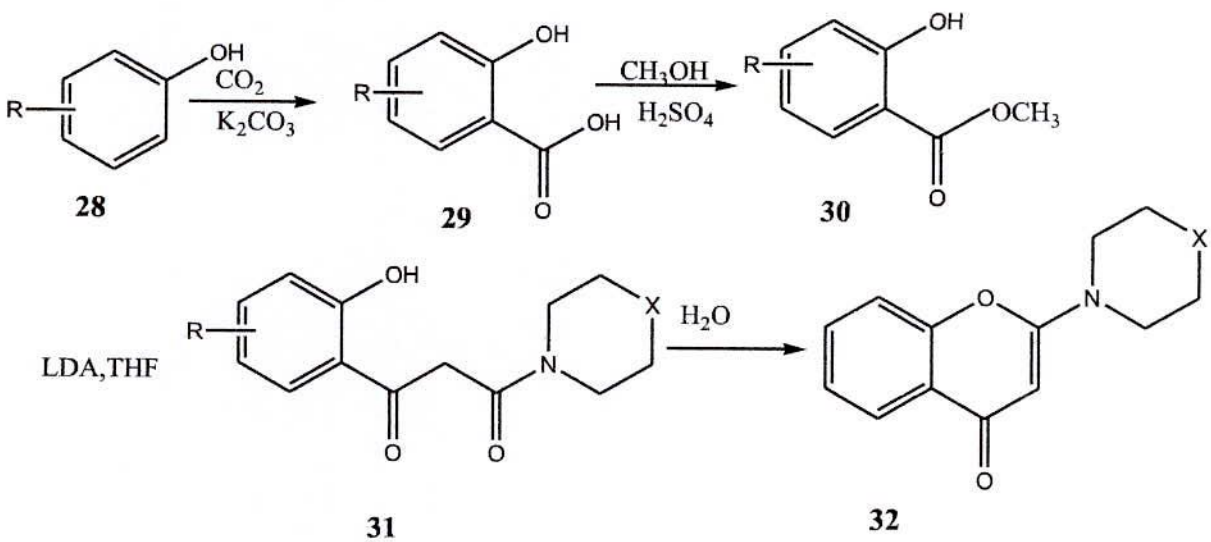


25 **26** **27**

Scheme. 2.11

2.3.6. Triflic anhydride as a catalyst

In 2005, Griffin *et al.* used triflic anhydride as a catalyst to construct the chromone ring (Scheme 2.12).Ring closure to the required chromones 32 was readily affected with triflic anhydride. Although theeffect of this catalyst was better, but higher prices due to trifluoroacetic anhydride, making its practical application being limited [56].



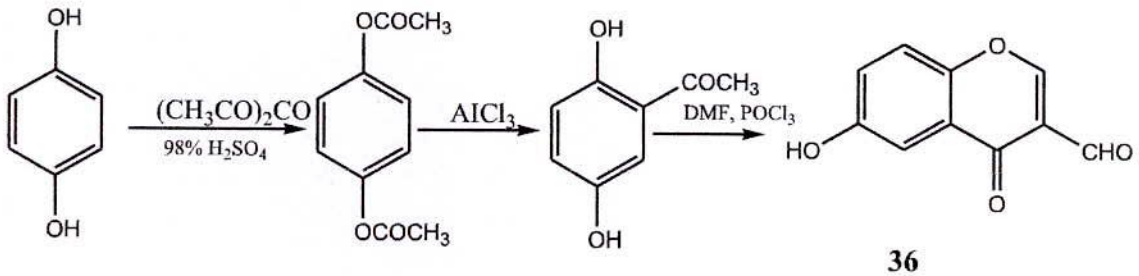
28 **29** **30**

31 **32**

Scheme. 2.12

2.3.7 Phosphorus oxychloride as a catalyst

In 2008, Yang and coworkers prepared the 6-hydroxy-3-carbaldehyde chromone via a Vilsmeier reaction in another way (Scheme 2.13). 6-hydroxy-4-chromone-3-carbaldehydes 36 were easily prepared by the reaction of 2,5-dihydroxy-acetophenone 35 with DMF in $POCl_3$ solution [57-58].



33 **34** **35** **36**

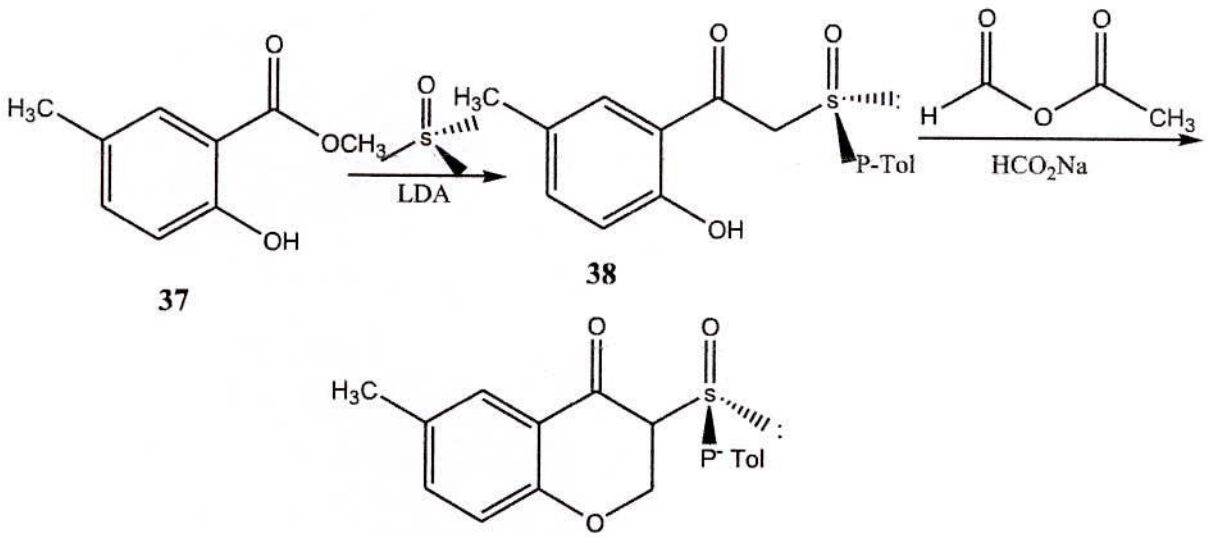
Scheme. 2.13

2.3.8 Base as catalyst in chromone ring closure

Although base as catalyst in the chromone ring closure is not common compared with acid, sometimes it can really bring some satisfactory results.

2.3.9 Sodium formate as a catalyst

In 2001, Wallace and coworkers reported the synthesis of enantiomerically pure (S)-2-methylchroman-4-one 39 based on the following procedure (Scheme 2.14). The formation of the chromone 39 was achieved conventionally using acetic formic anhydride and sodium formate, but this method is only applicable to compounds with ketosulfoxide [59-60].

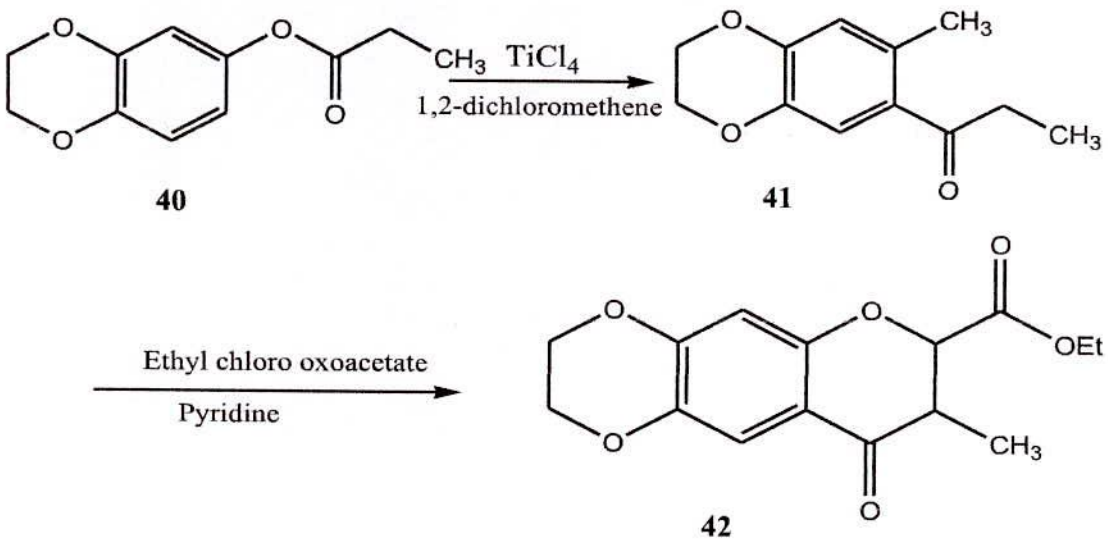


37 **38** **39**

Scheme. 2.14

2.3.10 Pyridine as a catalyst

In 2005, Lee *et al.* synthesized the chromone using pyridine as a catalyst in the ring closur(Scheme 2.15). This method of using pyridine as a catalyst was more suitable to acyl phenols andchloroacetyl carboxylic acid esters in the chromone ring closure [61].

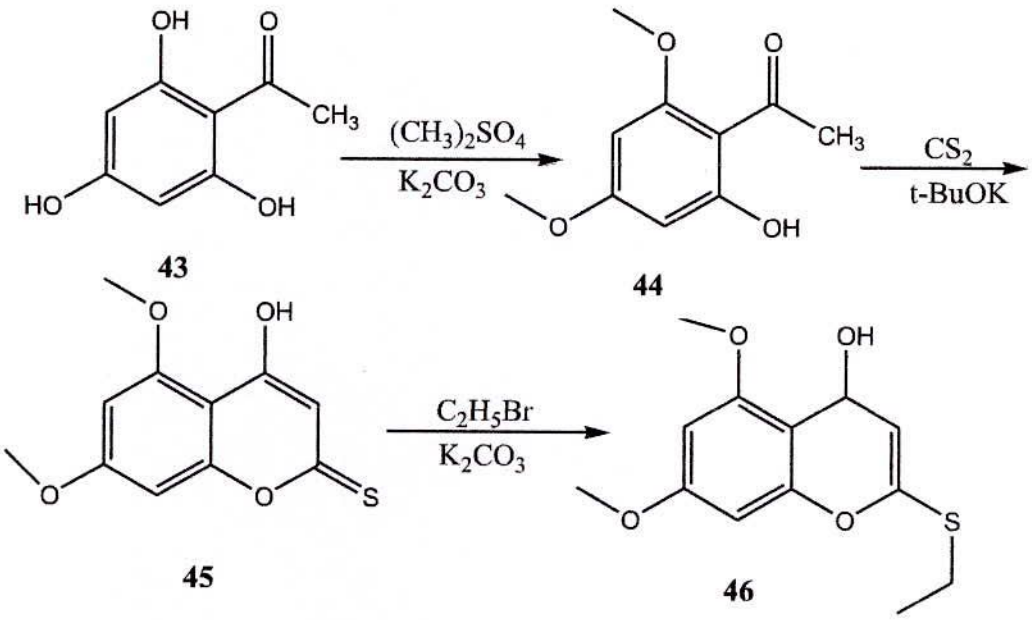


40 **41** **42**

Scheme. 2.15

2.3.11 Potassium tert-butoxide as a catalyst

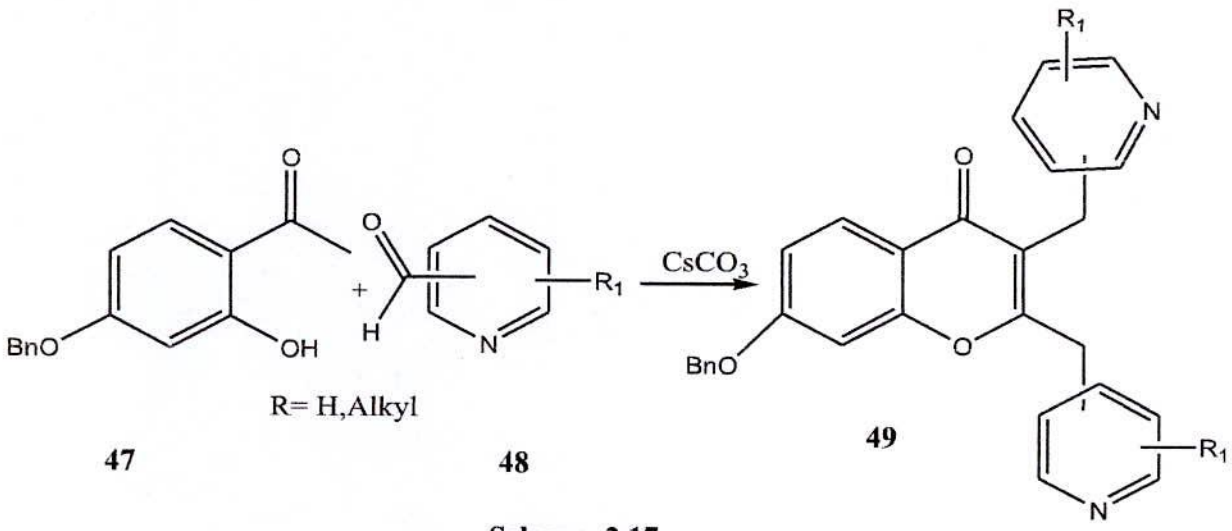
In 2007, Wu and coworkers prepared the chromone ring 46 using potassium tert-butoxide in the ring closure during their total synthesis 6-demethoxycapillarisin (Scheme 2.16). This reaction was very useful, which laid the foundation to expand the SAR of chromone ring with sulfur atom in the side chai [62].



Scheme. 2.16

2.3.12 Caesium carbonateas a catalyst

In 2008, Arai et al. described a practical and useful synthesis of heterocyclic substituted chromones (Scheme 2.17) and also developed a one-pot synthesis by Michael aldol reaction of chromone derivatives bearing heterocycle units. The 2,3-heterocyclic-substituted chromones 49 were obtained in one step, as shown in scheme 19, 4-benzyloxy-2-hydroxyacetophenone (47) reacted with heterocyclic aldehydes 48 to give 2,3-disubstituted chromone 49 in high yield under Cs_2CO_3condition [63].



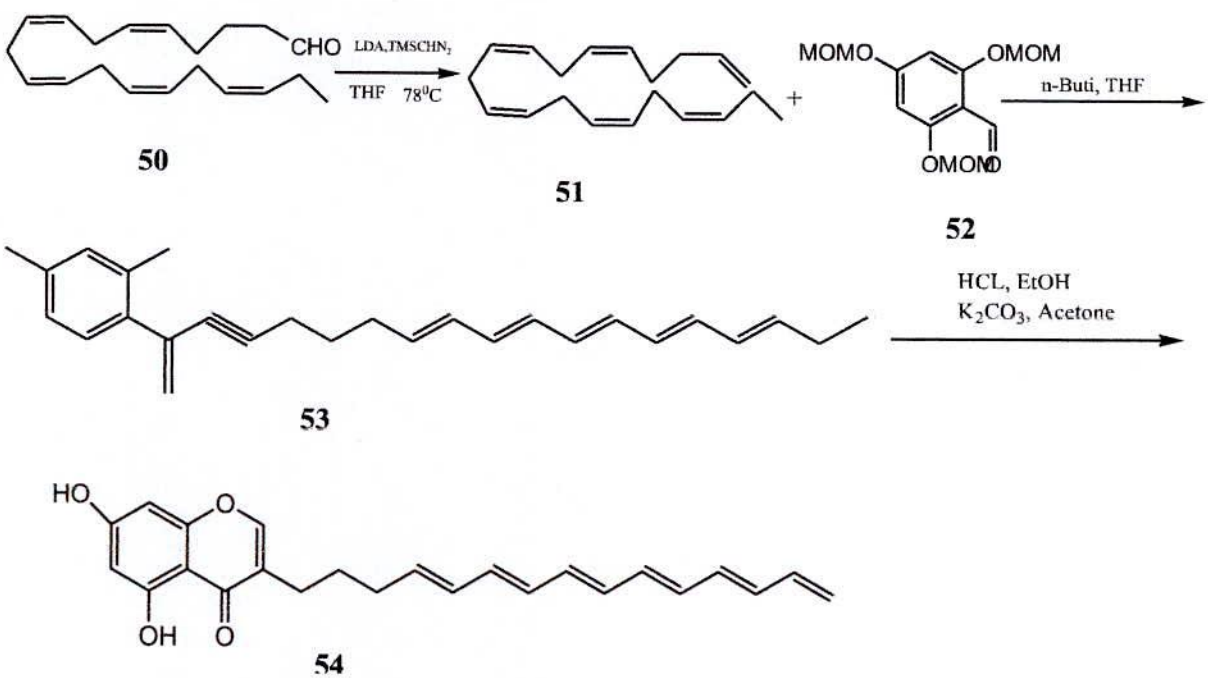
R= H,Alkyl

47 **48** **49**

Scheme. 2.17

2.3.13 Potassium carbonate as a catalyst:

In 2009, Anwar and Hansen used K_2CO_3 as a catalyst in the chromone ring closure during their first total synthesis of the marine natural product all-(Z)-5,7-dihydroxy-2-(4Z,7Z,10Z,13Z,16Znonadecapentaenyl) chromone 54 (Scheme 2.18). This reaction using phenol hydroxyl addition to the alkyne bond was relatively classical [64].



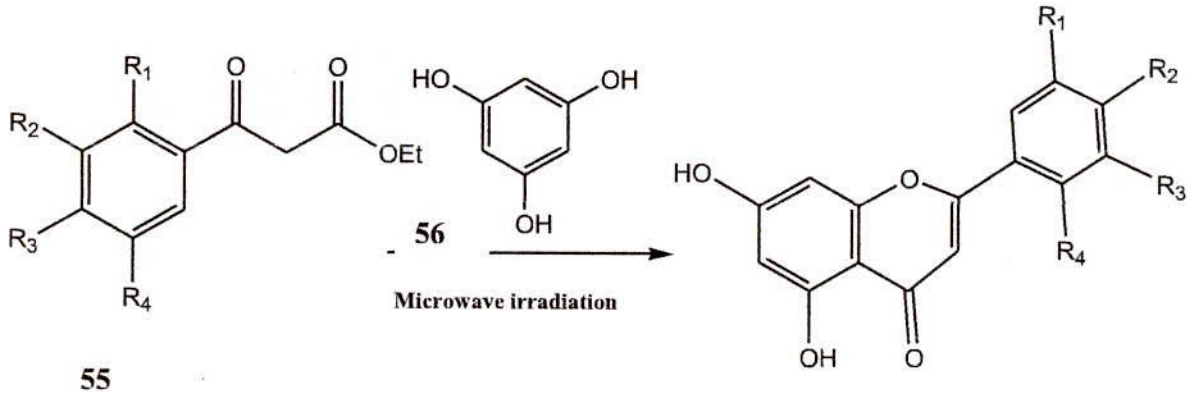
50 **51** **52** **53** **54**

Scheme. 2.18

2.3.14 Chromone ring closure under the microwave irradiation

Recently, microwave irradiation offers a considerable advantage over conventional heating because it results in substantial rate enhancements in a wide range of organic reactions. Cleaner reactions are also commonly achieved, together with improvements in yield and selectivity. The increasing demand for clean and ''green'' chemical syntheses has resulted in increased use of microwave irradiation, so there have been several recent reports, describing the application of microwave irradiation to the synthesis of flavonoids [65].

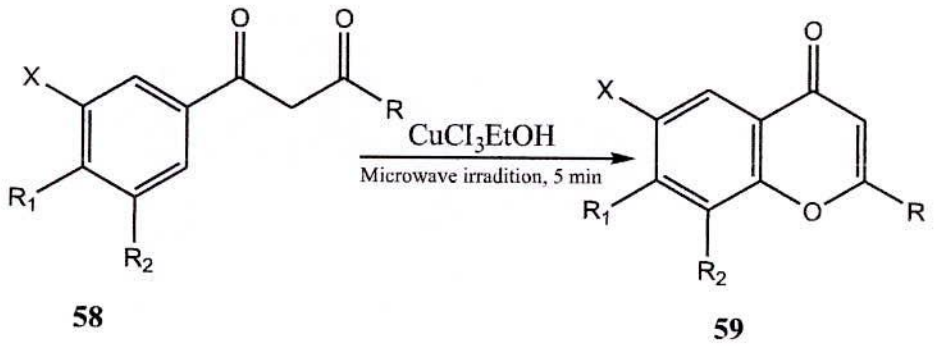
In 2005, Seijas et al. reported an eco-friendly direct solvent-free synthesis of functionalized flavones 57 under microwave irradiation (Scheme 2.19). This method was valid for flavones with or without substitutionin the B ring. Thus, the flavonoids were prepared from the corresponding ethyl benzoyl acetates 55 and phloroglucinol for 2–12 min of irradiation in 66–96% yields. The successful use of microwave irradiation in providing this rapid and direct route to flavones in comparison to classical procedures contributes to confirming the participation of specific effects in some microwave assisted organic synthes [66].



55 57

Scheme. 2.19

In 2005, Kabalka and Mereddy reported a facile microwave synthesis of functionalized flavones and chromones via the cyclization of 1-(2-hydroxyaryl)-3-aryl-1,3-propanedione (Scheme 2.20). In their study, the intermediate 1,3-propanediones 58 were synthesized in 5 min via dehydrative cyclization to the corresponding flavones and chromones 59 in ethanol, in the presence of $CuCl_2$ under microwave irradiation [67].



58 **59**

Scheme. 2.20

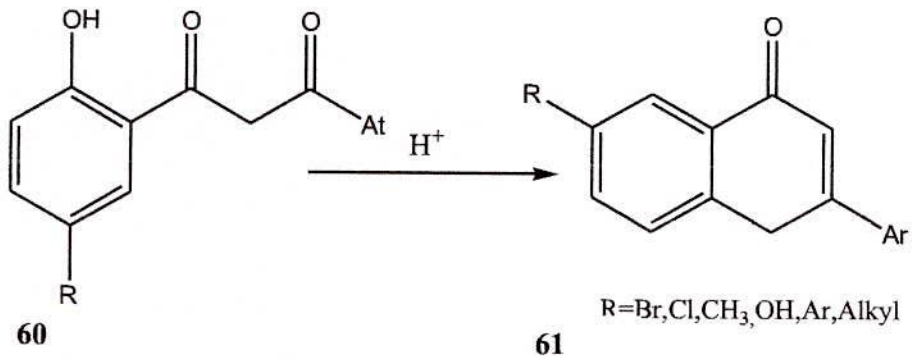
2.3.15 Chromone ring closure via solid-support

In recent years, solid-phase chemical reaction has appeared many advantages including good selectivity, high yield, simple operation, and no pollution, and some researcher has applied this method in chromone synthesis.

2.3.16 Via solid-support catalysts

In 2002, Blanco and coworkers studied the catalytic performance of phosphomolybdic acid (MPA) (H3PMo12O40.nH2O) and Phosphotungstic acid TPA ($H_3PW_{12}O_{40}$_nH_2O) (Scheme 2.21), both bulk or supported on silica (S), to obtain flavones and substituted chromones 61. The result showed that the conversion to flavones and substituted chromones was in general higher in homogeneous phase than that observed for the supported catalysts. Nevertheless, the use of the supported catalysts enabled an easy separation and recovery of the catalyst for its immediate reuse without any important decrease of the catalytic activity.

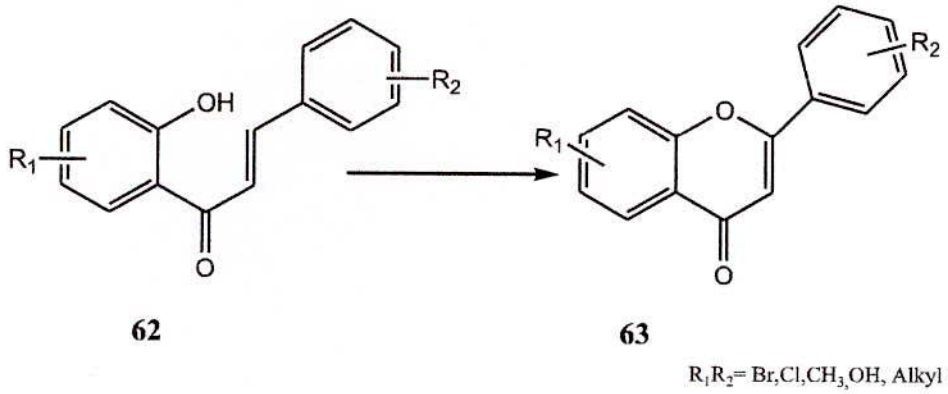
In addition, the unchanged starting material may be recycled to the reactor because it was almost quantitatively recovered and secondary products were not practically formed [68].



60 **61**

Scheme. 2.21

In 2005, van Lier and coworkers85 explored silica gel-supported InBr3 or $InCl_3$ (15–20 mol %) as a new solid-support catalysts for the facile and efficient oxidation, under solvent free conditions (Scheme 2.22), of 2'-hydroxychalcones 62 to yield the corresponding flavones 98 in >80% yield. The catalysts were easily prepared, stable, and efficient under mild reaction conditions [69].



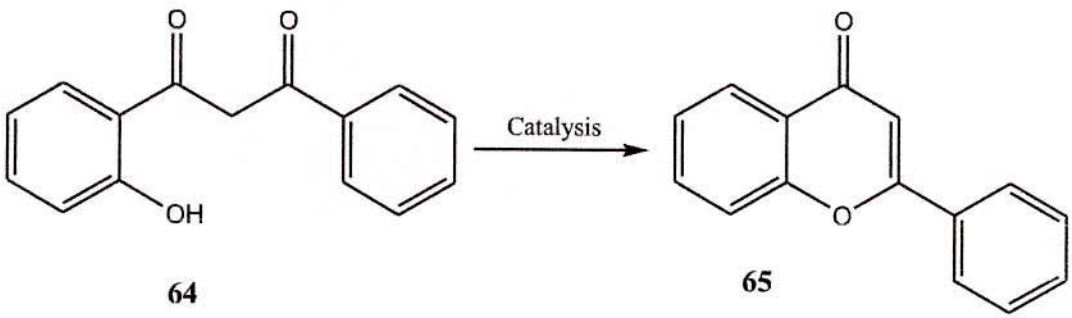
62 **63**

Scheme. 2.22

Trifluoromethanesulfonic acid (TFMS) is known to be a strong acid, and it is used in many organic reactions such as Friedel Crafts reactions, polymerization, Koch carbonylation, among others . However, the recovery of the triflic acid from the reaction mixture results in the formation of large amounts of waste [70].

In 2007, Romanelli and coworkers described the synthesis and characterization of TFMS supported on mesoporous titania using urea as a low-cost, pore-forming agent

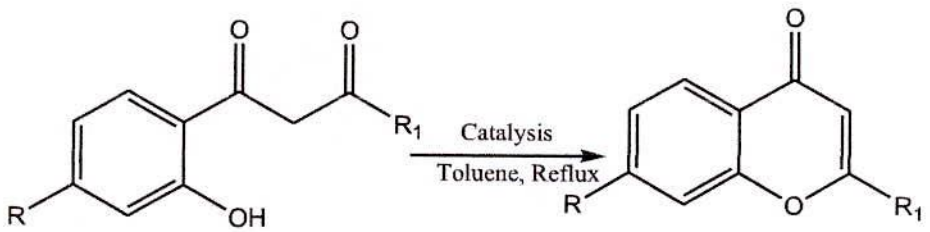
(Scheme2.23), via HCl catalyzed sol–gel reactions. The acidic characteristics of the solids were determined by potentiometric titration with n-butylamine. The use of these solid catalysts provided interesting yields in the cyclization reaction of 1-(2-hydroxyphenyl)-3-aryl-1,3-propanediones 64 to flavone 65, also leading to an easy separation and recovering of the catalysts for further use [71].



64 **65**

Scheme. 2.23

In 2009, Romanelli and coworkers also prepared the TFMSC1 and TFMSC2 catalysts by adsorption of TFMS on two activated carbons with different textural properties used as supports (Scheme 2.24). The TFMSC2 catalyst used as solid catalyst provided interesting yields in the cyclization reaction of 1- (2-hydroxyphenyl)-3-aryl-1,3-propanediones 66 to flavones and chromones 67, also leading to an easy separation and recovery of the catalysts for further use. Moreover, as a significant decrease of the catalytic activity was not observed, they can be recycled without any activity loss [33].



66

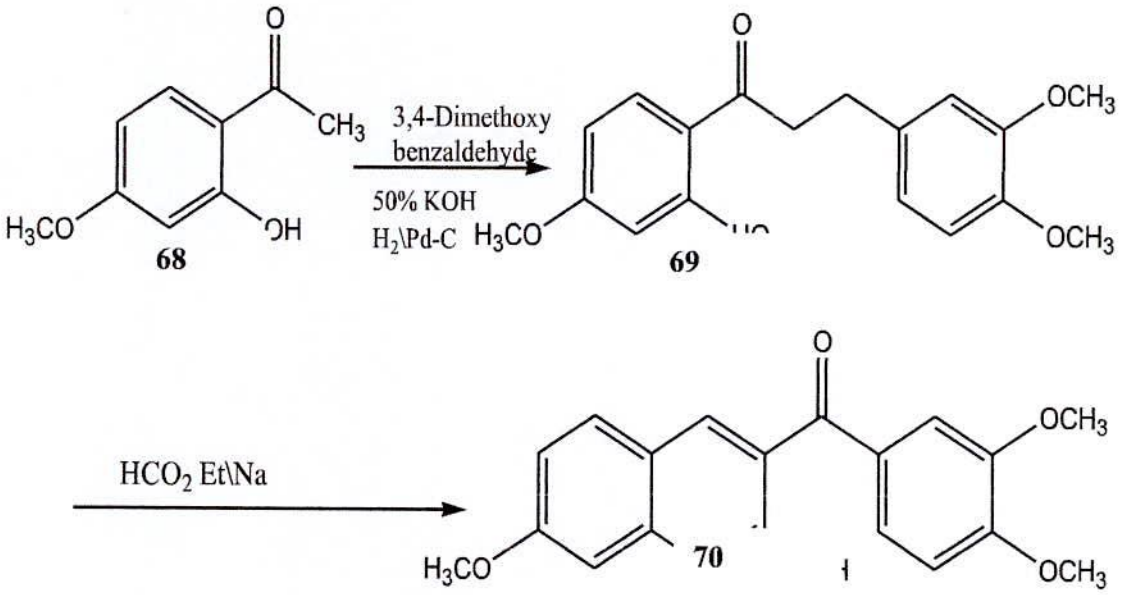
R=H,Cl,CH_3
R=Phenyl,1-Naphthyl,2-Naphthyl

67

Scheme. 2.24

2.3.17 Chromone ring closure through other methods

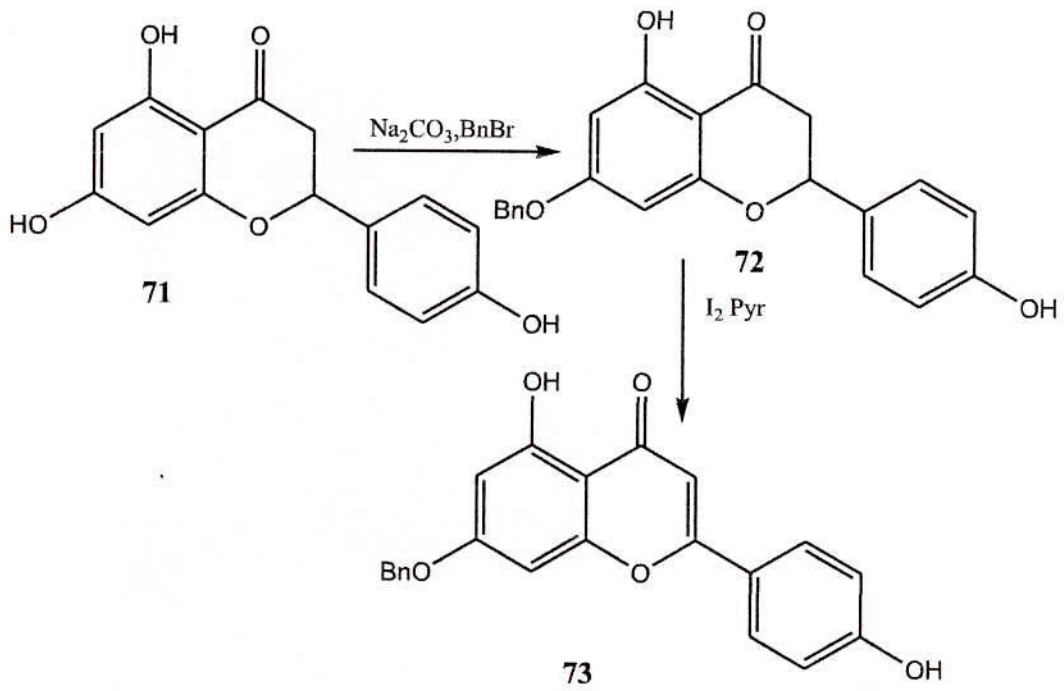
Besides the above acid catalyst, base catalyst, microwave irradiation, and solid-supported synthesis in ring closure, there are many other catalysts and reaction conditions in this chromone construction. The chromone 70 synthesized through a highly efficient procedure catalysted by sodium sand (Scheme 2.25). This reaction was not practical because the hot sodium sand was very dangerous during the reaction [73].



Scheme. 2.25

2.3.18 Through base-induced elimination

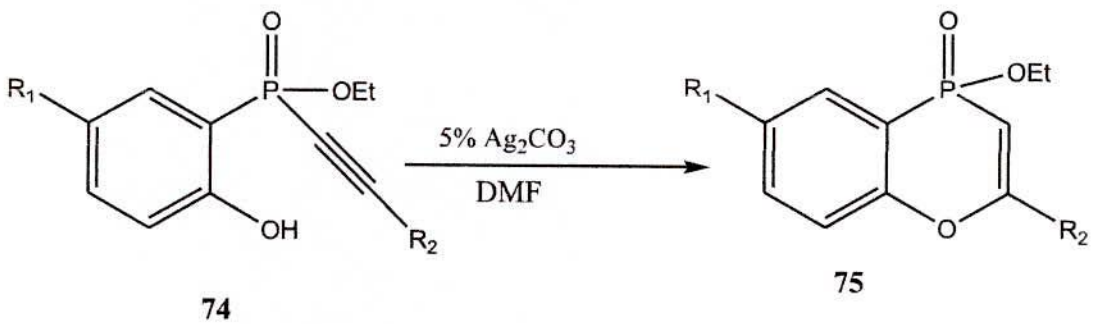
In 2009, Rizzacasa and coworkers described the synthesis of chromone 73 through iodination of naringenin followed by base-induced elimination (Scheme 2.26) [74].



Scheme. 2.26

2.3.19 Synthesis of phosphachromones

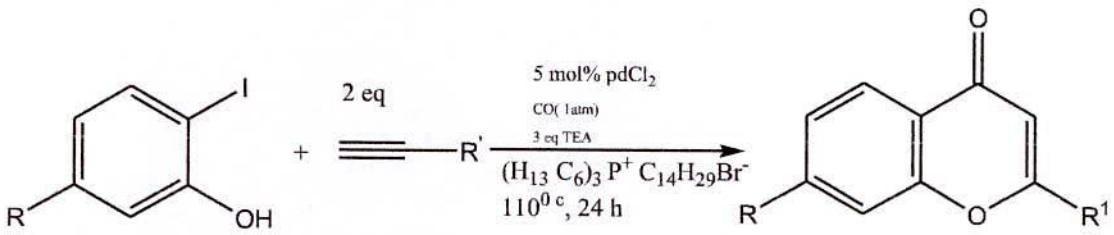
In 2008, Ding and coworkers reported a novel Ag_2CO_3-catalyzed cyclization reaction of o-hydroxyphenylethynylphosphinates 74 to phosphachromones 75 with high region selectivity and good yields (Scheme2.27), which provided an effective approach to synthesize the new kind of phosphorus heterocycles [75].



Scheme. 2.27

2.3.20 Recent Synthetic methods of chromones

A highly efficient and selective palladium-catalyzed ligand-free cyclocarbonylation reaction of oiodophenols 76 with terminal acetylenes under atmospheric CO pressure affords diversified chromones 77 in very good yields. The use of a phosphonium salt ionic liquid as the reaction medium enhances the efficiency of the cyclocarbonylation reaction (Scheme 2.28) [76].



76

R= H, CO_2 Me
R^1=Ar , Alkyl

Scheme. 2.28 77

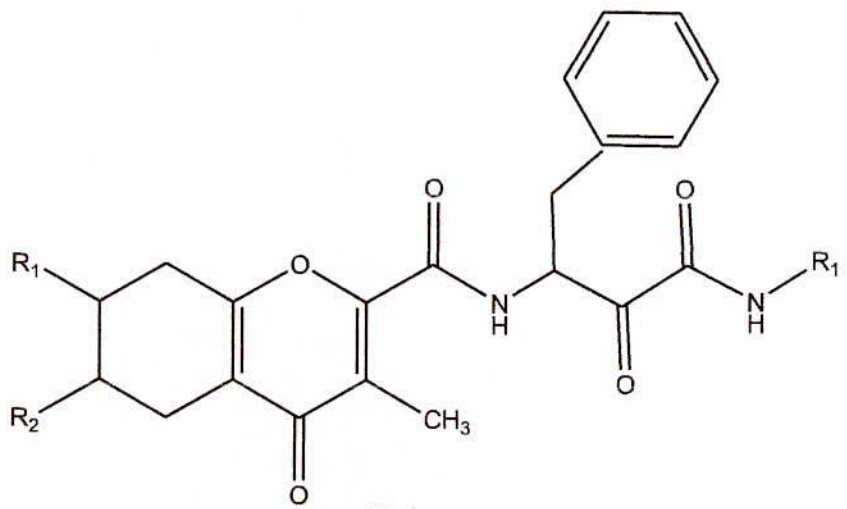
3.4 Biological Activity

Heterocycles play an important role in the design and discovery of new physiological/pharmacologically active compounds [77]. Chemically, chromones (4H-chromen-4-ones) are heterocyclic compounds with the benzo-c-pyrone framework. Molecules containing the chromone or benzopyranone ring have a wide range of biological activities. They have been shown to be tyrosine and protein kinase inhibitors109, [78] as well as anti-inflammatory, [79] antiviral, [80] antioxidant, [81-82]antihypertensive agents [80] and Chromone derivatives are also active at benzodiazepine receptors [83] In addition to this, they have been shown to be anticancer agents, [84] and possessing antimutagenic properties, [85]. Chromones may also have application in cystic fibrosis treatment, as they activate the cystic fibrosis transmembrane conductance regulator, [86]. Therefore, the vast range of biological effects associated with this scaffold has resulted in the chromone ring system being considered as a privileged structure, [87]. The main objectives of chromones syntheses are not only for the development of more diverse and complex bioactive compounds for biological activity and structure-activity relationship (SAR) studies but

also for other applications in Medicinal Chemistry, such as preparation of fluorescence probes, due to photochemical properties of chromones [88].

3.4.1 Antioxidant

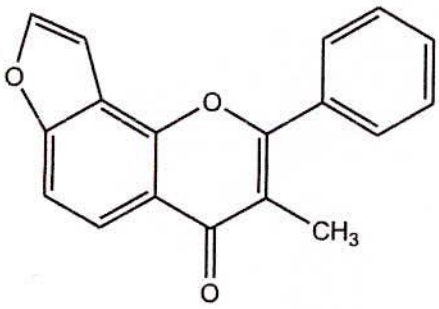
Lee and coworkers (2011), reported that new chromone carboxamide derivatives 78 were synthesized as conformationally constrained structural variants of MDL, to provide alternative Q- calpain inhibitors and antioxidant activities in DPPH scavenging and lipid peroxidation inhibitory effects [89].



78

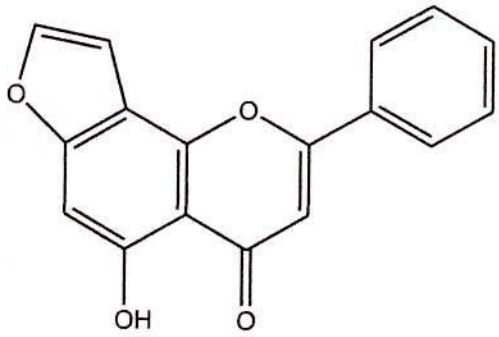
3.4.2 Antibacterial Activity

Pongaglabol exhibited activity against the bacteria Shigella dysenteriae, Streptococcus β-haemolyticus, and Staphylococcus aureus; the lowest concentration for inhibition of the first two types of bacteria amounts to 64 μg/ml [90] Methanol and ethyl acetate extracts from Pongamia pinnata plants in mixture with karangin 79 [91] exhibited antibacterial activity. An extract of flavonoids from Lonchocarpus montanus plants in dichloromethane, containing 19% of pongamol and 8% of lanceolatin B, exhibited activity against Staphylococcus aureus, whereas pongamol itself was active against Bacillus subtilis and Cladosporium cladosporioide [92].



Karangir

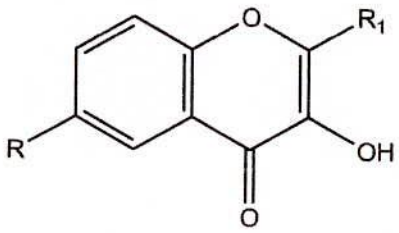
79



Pongaglabol

80

Gharpure, et al (2012) synthesized and evaluated 3-hydroxy-2-phenyl-4H-chromen-4-ones 81 asantibacterial activity. 3-Hydroxy-2-phenyl-4H-chromen-4-ones have been synthesized from appropriate 1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one. All compounds were evaluated for antibacterial activity against S. Aureus, B. Subtilis, E. Coli and P. Aerugenosa as well as fungi e.g. C. Albicans and A. Niger and good results were obtained as in comparison with the standards (Std. 1 = Gentamycin and Std. 2 = Clotrimazole) [93].



R=H,Cl R=C_6H_5 ,4-$CH_3C_6H_4$, 3,4-$(OCH_3)C_6H_3$,4-ClC_6H_4

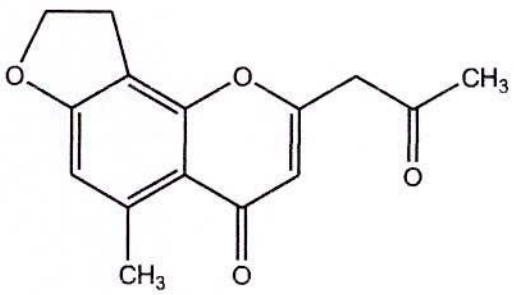
3-Hydroxy-2- phenyl-4H-chromene-4-ones

81

3.4.3 Anticancer Activity

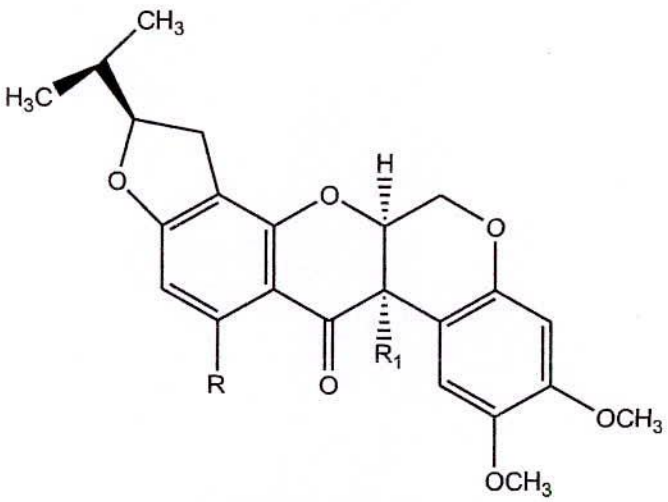
Furoaloesone 82, isolated from the plant Cape aloe, is capable to inhibit the growth of cancer cells of the Ehrlich ascitic carcinoma type. (−)-Sumatrol and (±)-villosinol from Lonchacarpus aff. fluvialisrind exhibited significant cytotoxicity against the cells of human oral epidermal carcinoma. Low toxicity in conjunction with high antitumor activity are

also known for the pyrano[2,3-e]indol-4(7H)-one system. Such properties were found, for example, For compound 12-amino-2-phenylpyrano[2,3- a]-acridin-4-one 83 (APPA), investigations of the antiproliferative activity in relation to tyrosine kinase in DHER cells showed that for this compound IC50 = 1.9, while for acronycine IC50 = 3.6 nmol/l. The product APPA also displayed inhibiting activity against more than 60 lines of cancer cells; the IC50 values varied in the range of 0.1-1.4 nmol/l. The best results were obtained for leukemia [94-95].

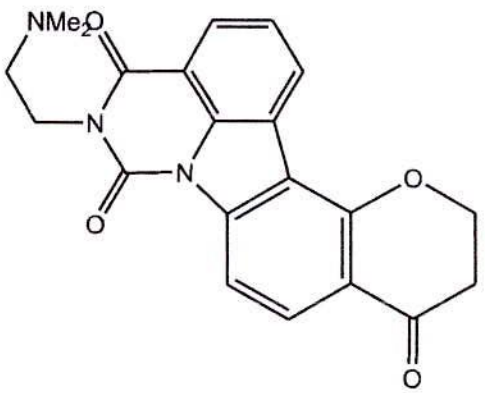


Furoaloesone

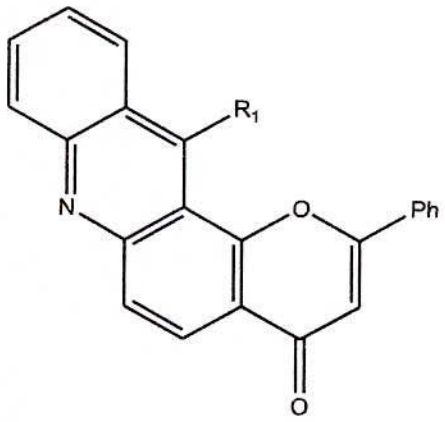
82



R=OH, R_1=H
R=OH,R_1=OH



(-) Sumatrol(±) - Villosinol

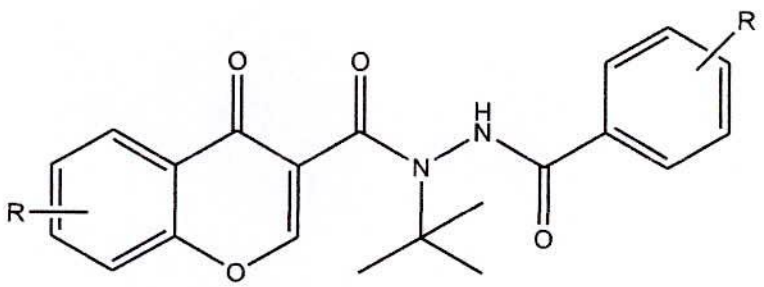


12-amino-2-phenylpyrano
[2,3-a]-aciridin-4-one(APPA)

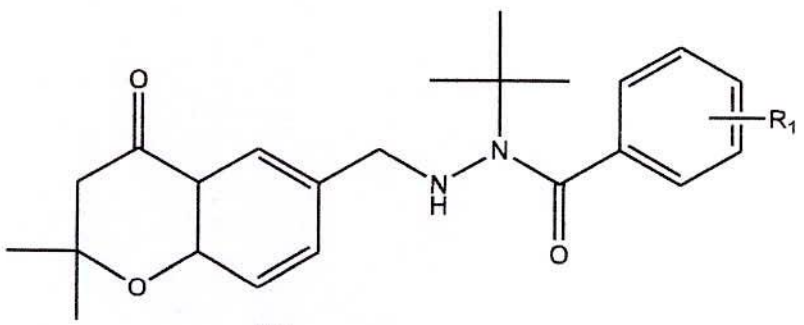
83

3.4.4 Insecticidal Activity

Zhao and coworkers prepared a series of chromanone and chromone analogues of diacylhydrazine derivatives. Some of the chromanone analogues exhibited a good insecticidal activity against Mythima separate at the dosage of 500mg/L [96].



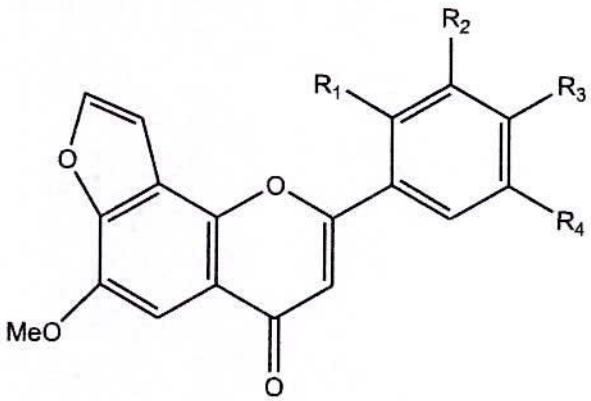
84



85

3.3.5 Antiviral Activity

The methyl ether of pongol 86 isolated from the plant Millettia erythrocalyx, exhibited activity against both types of herpes virus HSV-1 and HSV-2138. Activity against human immunodeficiency virus (HIV) and herpes virus was found in alkaloids from the Schumanniophyton plants. The presence of the piperidine ring and unsubstituted hydroxylgroups in their molecules is responsible for the activity against HIV [97-98].



$R_1 = R_2 = R_3 = H$, $R_1 = OH$

86

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 measure from our laboratory, FTIR spectra and NMR was carried out at CARS in Jahangirnagar University and Hiroshima University in Japan.

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 (950 mL) was poured into the round-bottomed flask and refluxed for 40 minutes. Then the mixture was distilled and ethanol was collected (bp 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 Reagent

3.3.1 Anhydrous Zinc chloride

Commercial grade anhydrous zinc chloride was placed in a porcelain basin and heated over a small flame. The salt first liquefied, steam was evolved the mass solidified as soon as most of

the water had been driven off. To remove the residual water the solid has carefully heated with large flame until the solid just melted. Overheating was avoided, which was recognized by the evolution of combustible gases. The fused salt was allowed to solidify in desiccators and added to the reaction mixture.

3.4 Experimental Techniques Employed

3.4.1 Chromatographic Technique (Thin layer chromatography)

Chromatography is a separation process, which depend on the differential distributions of the component of a mixture between a mobile bulk phase and an essentially thin film stationary phase.

When this stationary phase remain in the form of a thin layer adhering to a suitable from of backing material over which the mobile phase is allow to ascent by capillary action, the technique is called thin layer chromatography (TLC). The most commonly used stationary phases, which are available in different grades specially prepared for TLC use, include silicagel, alumina, kieselguhr and cellulose powders.

Preparation of plates

In this technique the glass plates were cleaned by detergent to remove the greasy material and then dried. The plates were coated with silica gel. The TLC plates (2.5 cm× 6.0 cm) were prepared by dipping the plates into sullary made of UV active silica gel (Merch, UV action) (30 g) in distilled water (60 mL) the thickness of the layer all over the plates were kept uniform to a value of 0.25 mm and dried at room temperature. The plates were activated by heating at 110^0C for 10-12 hours in an oven.

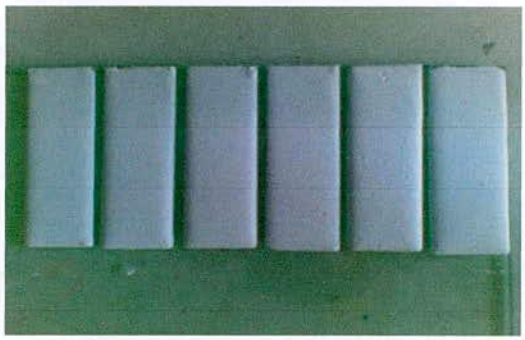


Fig: 3.1: Sample of TLC Plat

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.

(1) (2)

Fig 3.2: (1) TLC tank & (2) 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.

3.5 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.

3.6 Application of sample on the plate

Sample can be applied to thin layer by platinum loop, capillary tube, micropipette or Hamilton syring. But considerably more skill and care are needed that a hole is not produced on the film.

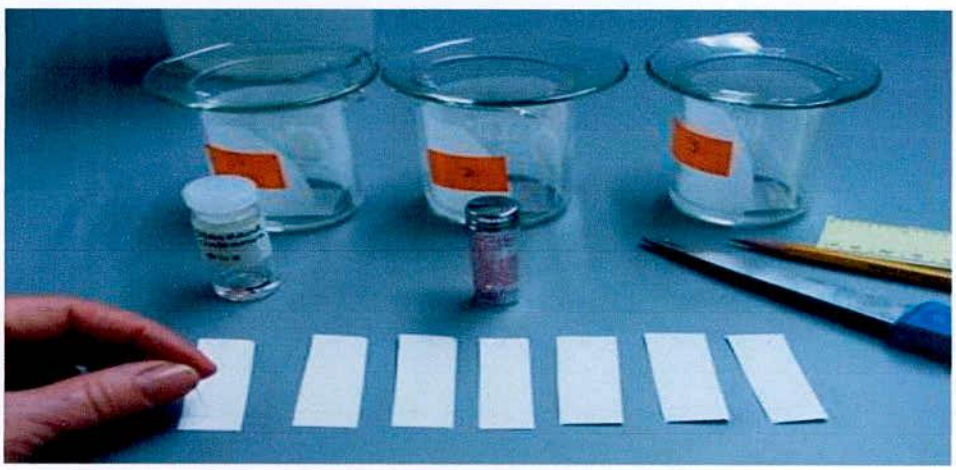


Fig 3.3: process of spotting

3.7 Solvent Systems

The solvents of different polarity used for TLC are given below:

1. N-hexane
2. N-hexane: DCM (in different ratio)
3. Dichloromethane: Ethanol(EtOH) (in different ratio)
4. Ethyl acetate: N-hexane
5. (EtOH): MeOH (in different ratio)

3.8 Development of plates:

When the sample was spotted on the film of plated, it was developed in ascending process. Usually plates were placed in one tank. The plates were put in the 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.

3.9 Location of Spots:

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.

3.10 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.

3.11 The R_f value:

The retarding factor (R_r value) of any compound seen on a TLC plate was calculated according to the following equation

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

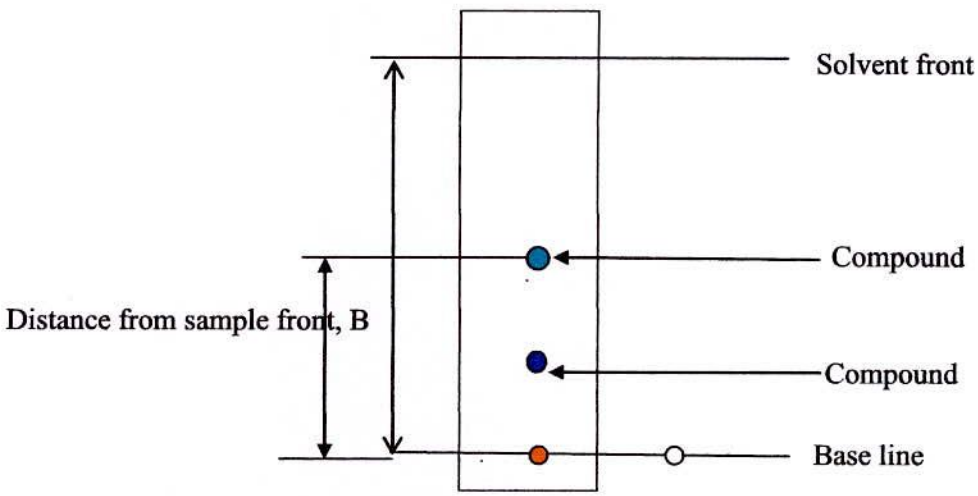


Fig: 3.4: A Plate for the calculation of R_f value

3.12 Recrystallization

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

3.13 Spectroscopic Techniques

Ultraviolet and visible spectra:

Infra-red-spectra: Infra-red spectra of the samples were recorded on the SHIMADZU-IR-400 infra-red spectrophotometer within the range of 4000-400cm^{-1}.The spectra for solid samples were recorded as KBr pellets.

^{1}H NMR spectra:

NMR spectra of the sample were recorded on a 400 MHz NMR spectra. The solvent used were d_6 DMSO and $CDCl_3$. TMS was used as an internal standard.

3.14 Rotary evaporator

All evaporations were carried under reduced pressure using rotary vacuum evaporator; the bath temperature was not exceeding 40-50° C.

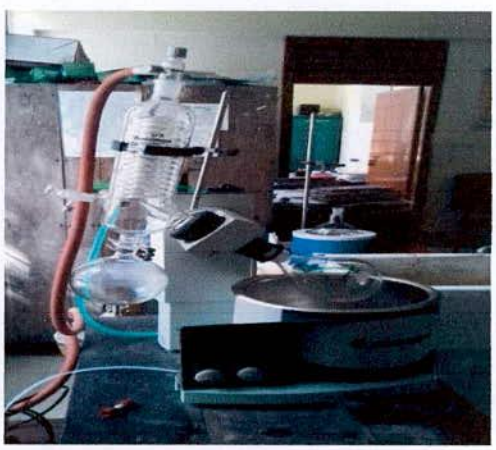


Figure: 3.4.1: Rotary evaporator

3.15 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
δ	Chemical shift
TMS	Tetra methyl silane
D_6	Deuterated Dimethyl sulfoxide

3.17 Melting point apparatus

Fig 3.5: Melting point apparatus

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

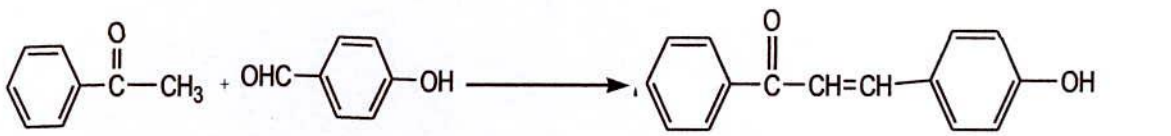
3.18 Preparation of substituted chalcones:

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

Procedure

4-hydroxyl benzaldehyde (0.01 mol) and acetophenone (0.01 mol) 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 pure product was 184-185° C. The yield of the pure 3-(4-Hydroxy-phenyl)-1-phenyl-propenone 9.4 g.

Reaction scheme

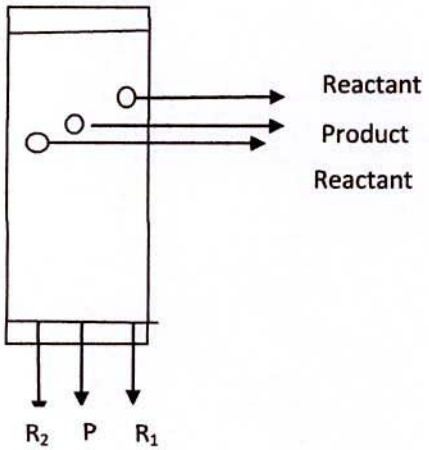


Acetophenone 4-hydroxy benzaldehyde 3 (4-hydroxy phenyl) -1-phenyl -propenone

Characterization and structure determination of the product

The structure determination of the product was 3- (4-hydroxy phenyl)- 1-phenyl-propenone given Fig 3.1 .The product of the color was yellow and the melting point of the pure product was 184-185°C.

Behaviour in TLC



R_1= Acetophenone

P= Product

R_2= 4-Hydroxy benzaldehyde

R_f value= 0.45

Spectral properties

IR Spectrum

The IR spectrum of the product run as KBR pellet showed where absorption band V_{max} in cm^{-1} which as assigned as:

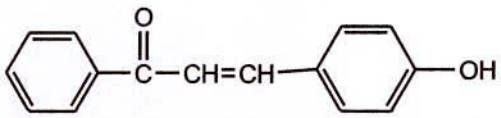
3268, 3050, 1650, 1602, 936,836

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

The ^{1}H NMR spectrum (Fig 3.1.1) of the compound in $CDCl_3$ gave the following signals (δ value) using TMS as an internal standard.

^{1}H NMR (400Mz $CDCl_3$) 8.036 (d, J = 8.8 Hz, 2H, Ar), .7.807 (d, J = 15.6 Hz, 1H), 7.509-7.609 (m, 5H, Ar), 7.435 (d, J=16 Hz, 1H), 6.926 (d, J= 8.8Hz, 2H), 5.820 (s, OH)

On the basis of physical properties, chemical behavior and spectral properties the following fig (3.1.1) has been assigned to this product



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

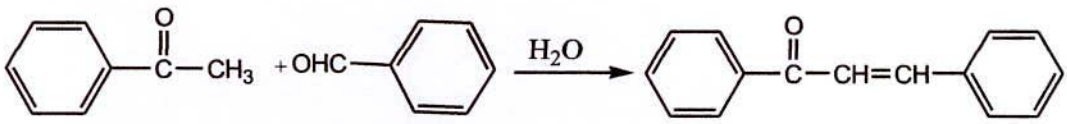
Figure: 3.1.1

3.19 Preparation of 1,3 diphenyl-propanone

Procedure

Acetophenone (3.60 g) and Benzaldehyde (3.18 g) were dissolved in ethanol(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 (5 g 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; n-hexane, ethanol). 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. The melting point of the pure product was 46-47°C. The yield of the pure benzylidene acetophenone 3.5 g.

Reaction scheme:

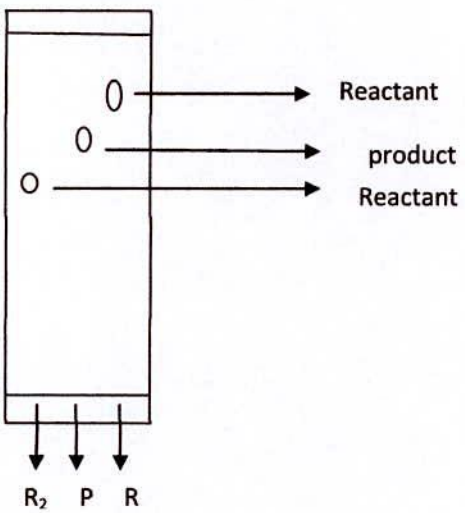


Acetophenone Benzaldehyde 1,3-diphenyl-propane

Characterization and structure determination of the product:

The structure determination of the product 1,3 di-phenyl propanone given Fig 3. 2. The product of the color was white color and the melting point of the pure product was 42°-43°C.

Behaviour in TLC



R_1= Acetophenone

P= Product

R_2=Benzaldehyde

R_F value= 0.40

Spectral properties

IR Spectrum

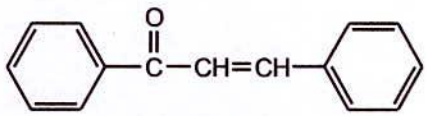
The IR spectrum of the product run as KBR pellet showed absorption band *V*max in cm-1 which as assigned as:

3050, 2926, 1661,1603, 1447, 1217, 746.

^{1}H NMR spectrum

^{1}H NMR (400 MHz, $CDCl_3$): 8.054 (d, J = 7.2 Hz,2H,Ar), 7.856 (d, J = 16 Hz, 1H), 7.565 (d, J=16, 1H), 7.440-7.688 (m, 8H, Ar

On the basis of physical properties, chemical behavior and spectral properties the following structure has been assigned to this product



1,3-diphenyl-propane

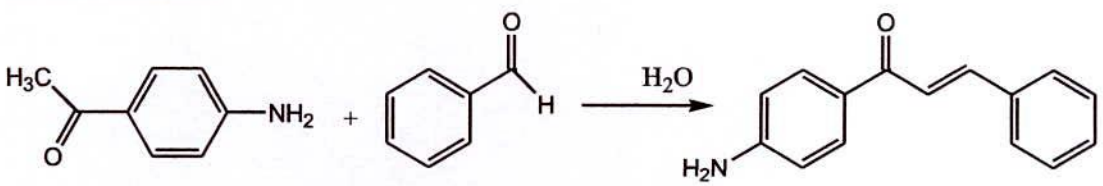
Figure: 3.1.2

3.20 Preparation of 1-(4-amino-phenyl)-3-phenyl-propenone

Procedure:

4-amino acetophenone (0.01 mol) and benzaldehyde (0.01 mol) was dissolved in 100 mL ethanol. After 40 mins, to this solution, NaOH (40%, 10mL) 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 hours. 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 litmus. It was crystallized from ethanol.

Reaction Scheme

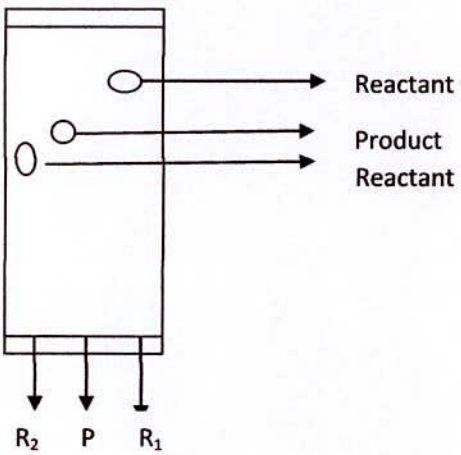


4-amino acetophenone Benzaldehyde 1-(4-Amino-phenyl)-3-phenyl-propenone

Characterization and structure determination of the product

The structure determination of the product was 1-(4-amino-phenyl)-3-phenyl-propenone given Fig 3.1.3 .The product of the color was yellow color and the melting point of the pure product was 142°-143°C.

Behaviour in TLC



R_1= Benzaldehyde

P= Product

R_2= 4- amino acetophenone

R_f value= 0.45

Spectral properties

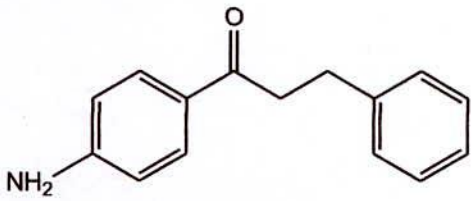
IR Spectrum

The IR spectrum of the product run as KBR pellet showed absorption band *V*max in cm^{-1} which as assigned as:
3362, 3000, 1662, 1229.

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

The ^{1}H NMR spectrum (Fig 3.3) of the compound in $CDCl_3$ gave the following signals (δ value) using TMS as an internal standard.
^{1}H NMR 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 of physical properties, chemical behavior and spectral properties the following structure has been assigned to this product



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

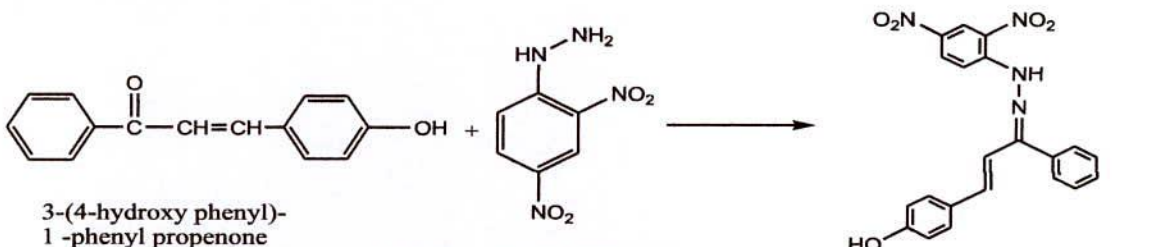
Figure:3.1.3

3.21 Preparation of 4-{3-[(1-Methylene-4-nitro-but-2-enyl)-hydrazono]-3-phenyl-propenyl} - phenol

Procedure:

3- (4-hydroxy phenyl)- 1-phenyl-propenone (0.01 mol) was dissolved in 100 mL ethanol. Then 2,4 dinitro phenyl hydrazine 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 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). At the end of this reaction precipitation occurred. Where by precipitation occurred. The precipitate was filtered off and dried. The R_f value of the product was 0.45 g.

Reaction Scheme



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

2,4 dinitrophenyl hydrazine

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

Characterization and structure determination of the product

The structure determination of the product was 4-{3-[(1-Methylene-4-nitro-but-2-enyl)-hydrazono]-3-phenyl-propenyl}- phenol given Fig 3.1.4.The product of the color was black and the melting point of the pure product was 142°-143°C.

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

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

H_1 NMR (400Mz $CDCl_3$) 8.342-8.371 (m, 1H, Ar), 8.126 (d, J =9.2 Hz, 1H, Ar), 8.033 (d, J = 7.2 Hz, 2H, Ar), 7.799 (d, J = 15.6 Hz, 1H), 7.351-7.686 (m, 7H, Ar), 7.178 (d, J= 16.4, 1H, NH), 6.835-6.986 (m, 2H), 6.517 (d, J= 16.4, 1H), 5.476 (s, 1H, OH).

On the basis of physical properties, chemical behavior and spectral properties the following structure has been assigned to this product

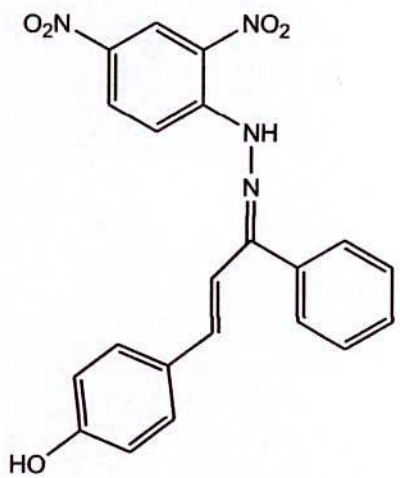


Figure: 3.1.4

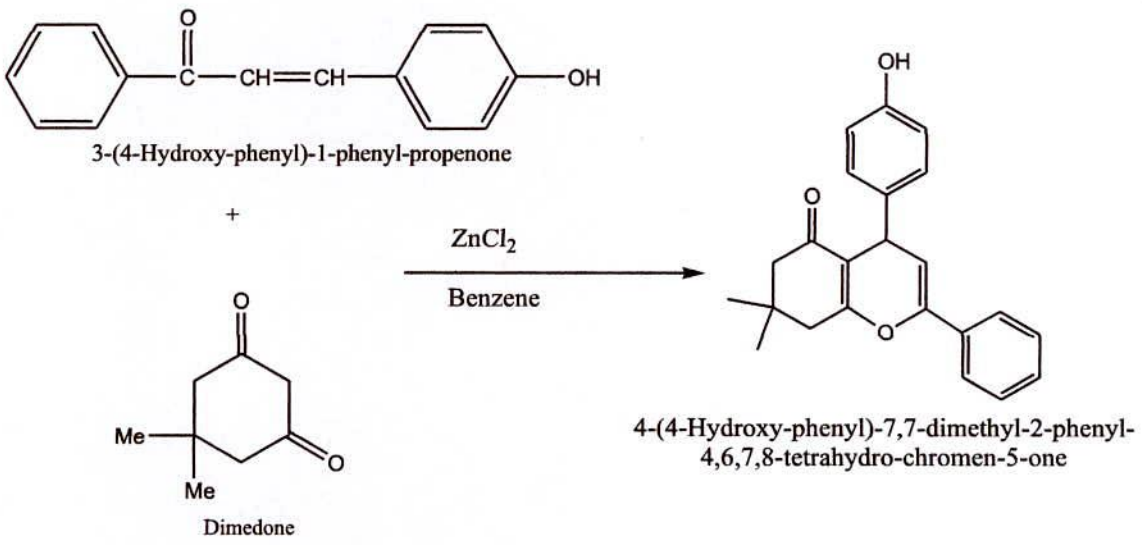
3.22 Preparation of chromenes derivative

3.22.1 Reaction of 3-(4-Hydroxy-phenyl)-1-phenyl-propenone with dimedone in presence of zinc chloride catalyst.

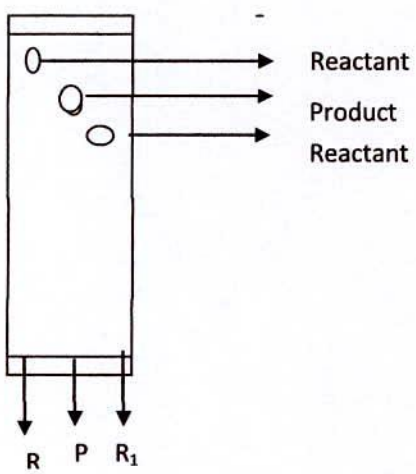
Procedure:

A mixture of dimedone (0.35 g, 0.0025 mol), chalcon (0.56g, 0.0025 mol) and zinc chloride (0.05 g) was taken in a round bottom flask (250 mL). To this benzene (32 ml) was added. The mixture was then reflux for 72 hours with constant stirring. The progress of the reaction was followed by TLC on silica plates (eluting solvent ethyl acetate: n-hexane). When both reactants disappeared, the reaction mixture was neutralized by 0.01M HC. Where by precipitation occurred. The precipitate was filtered off and washed with cold water until the washings were neutral to litmus. The amount of crude product was 3.5 g.

Reaction Scheme:



Behavior in TLC



R_1=Dimedone

R_2=Chalcone

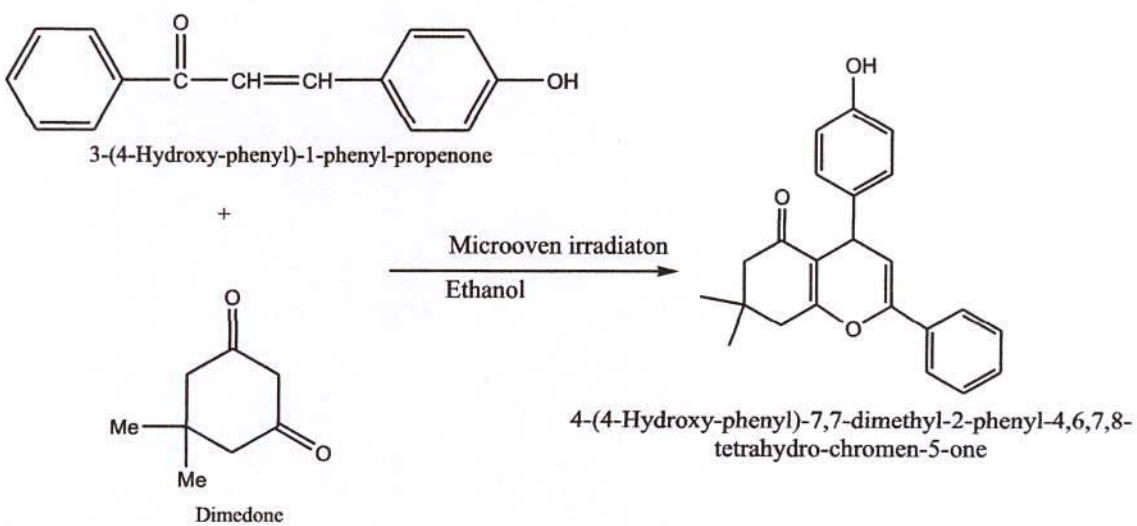
P= product (impure)

3.23. Microwave irradiation method

In a 250 mL conical flask an equimolar mixture of diamedone (.0025 moL) and chalcon (0.0025 mol) were dissolved in 25ml ethanol. The mixture was irradiated with microwave at 350w power for several minutes and progress of reactions was followed by TLC on silica gel plate (eluting solvent ethyl acetate: n-hexane; 40:60). The reaction mixture was cooled and the solid was separated out by filteration and recrystallized from n-hexane and ethyl acetate. The purity of the product was checked by TLC. The R_f value of the product was 0.76. The weight of the product was 0.35g.

Fig 3.6: Micro Oven

Reaction Scheme:

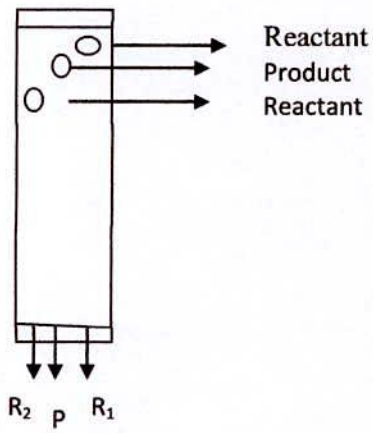


Characterization and structure determination of the product

The structure determination of the product was 4-(4-Hydroxy-phenyl)-7,7-dimethyl-2-phenyl-4,6,7,8-tetrahydro-chromen-5-one given Fig 3.1.5. The product of the color was white the melting point of the pure product was 162°-165°C. The compound was soluble in $CDCl_3$, DMSO, n-hexane, ethylacetate and absolute alcohol.

Behaviors in TLC

The product showed a single spot on TLC (Stationary phase: silica gel ,eluting solvent $CHCl_3$



P= Product

R_1= Dimedone

R_2= Chalcone

R_f= 0.76

Spectral properties

IR Spectrum

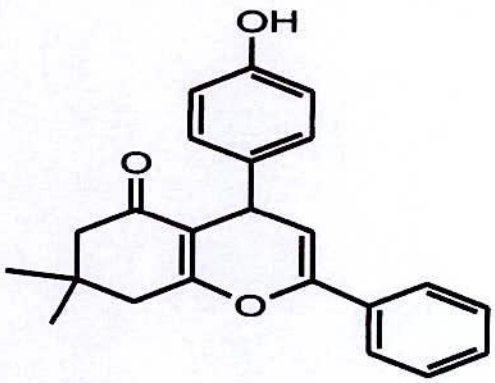
The IR spectrum of the product run as KBR pellet showed absorption band *V*max in cm^{-1} which as assigned as:
3326, 2980, 1666, 1620.

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

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

^{1}HNMR (400Mz $CDCl_3$) 11.910 (s, 1H, OH), 6.636-7.286 (m, 9H, Ar), 5.490 (s, 2H), 2.314 (s, 2H, CH_2), 2.256 (s, 2H CH_2), 1.093 (s, 6H, CH_3).

On the basis of chemical behavior and spectral properties the following structure has been assigned to this product



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

Figure: 3.1.5

3.4 Antimicrobial Activity

3.4.1 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 12 µg/µL and 300 µg/disc. Ciprofloxacin and miconazol were used as standard drug at a concentration level of 25 µg/disc and 50 µg/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.

3.4.2 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 [99].

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

3.4.3 Test materials of Sample

1. (4-hydroxy phenyl)- 1-phenyl-propenone
2. 4-(4-Hydroxy-phenyl)-7,7-dimethyl-2-phenyl-4,6,7,8-tetrahydro-chromen-5-one
3. 4-{3-[(1-Methylene-4-nitro-but-2-enyl)-hydrazono]-3-phenyl-propenyl}-phenol

3.4.4 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*).

Table 3.1: List of Test Bacteria and fungi

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

3.4.5 Methods

3.4.6 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.

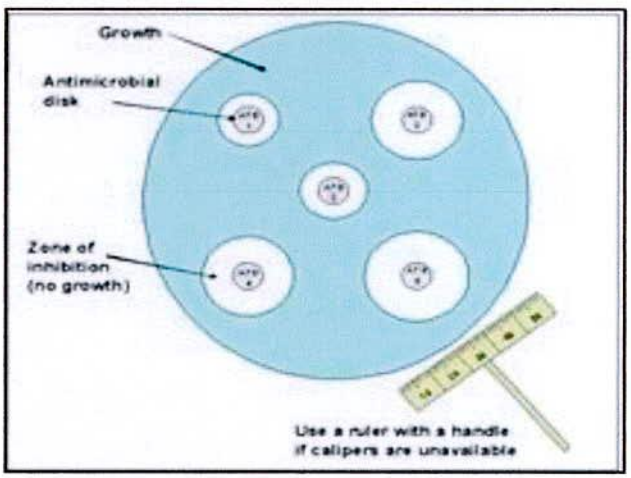


Fig 3.7: Inhibition Zone measurement

3.4.7 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 mLof 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 25 mL 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 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.

Fig 3.8: 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.

Fig 3.9: Application of discs

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. 25 µl of solution was injected on each disc.

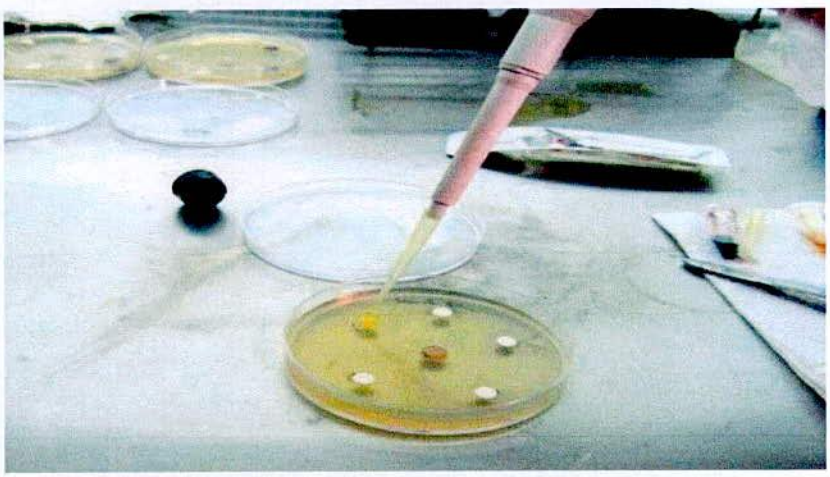


Fig 3.10: Application of Samples on the discs

CHAPTER IV

RESULTS AND DISCUSSIONS

CHEAPTER IV

Result and discussion

4.1 Reaction of 4- Hydroxy benzaldehyde and acetophenone

The reaction of 4- hydroxy benzaldehyde with acetophenone in presence of ethanol at room temperature was given a crystalline solid, m.p.184-185°C. The R_f value of the product was found to be 0.58. On [eluting solvent; ethyl acetate, n-hexane= 2:3].

Spectral properties

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

Table 4.1: IR value of compound I Vmax in cm-1

Absorption bands, *V*max in cm^{-1}	Group present
3268	OH group (H-bond, intermolecular)
3050	Aromatic C-H
1650	=C=O in conjugation with =C=C=
1602	=C=C in conjugation with =C=O and C=C stretch of phenyl
936, 836	=C-H bending of phenyl

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.

H_1 NMR (400Mz $CDCl_3$) 8.036 (d, J = 8.8 Hz, 2H, Ar), .7.807 (d, J = 15.6 Hz, 1H), 7.509-7.609 (m, 5H, Ar), 7.435 (d, J=16 Hz, 1H), 6.926 (d, J= 8.8Hz, 2H), 5.820 (s, OH)

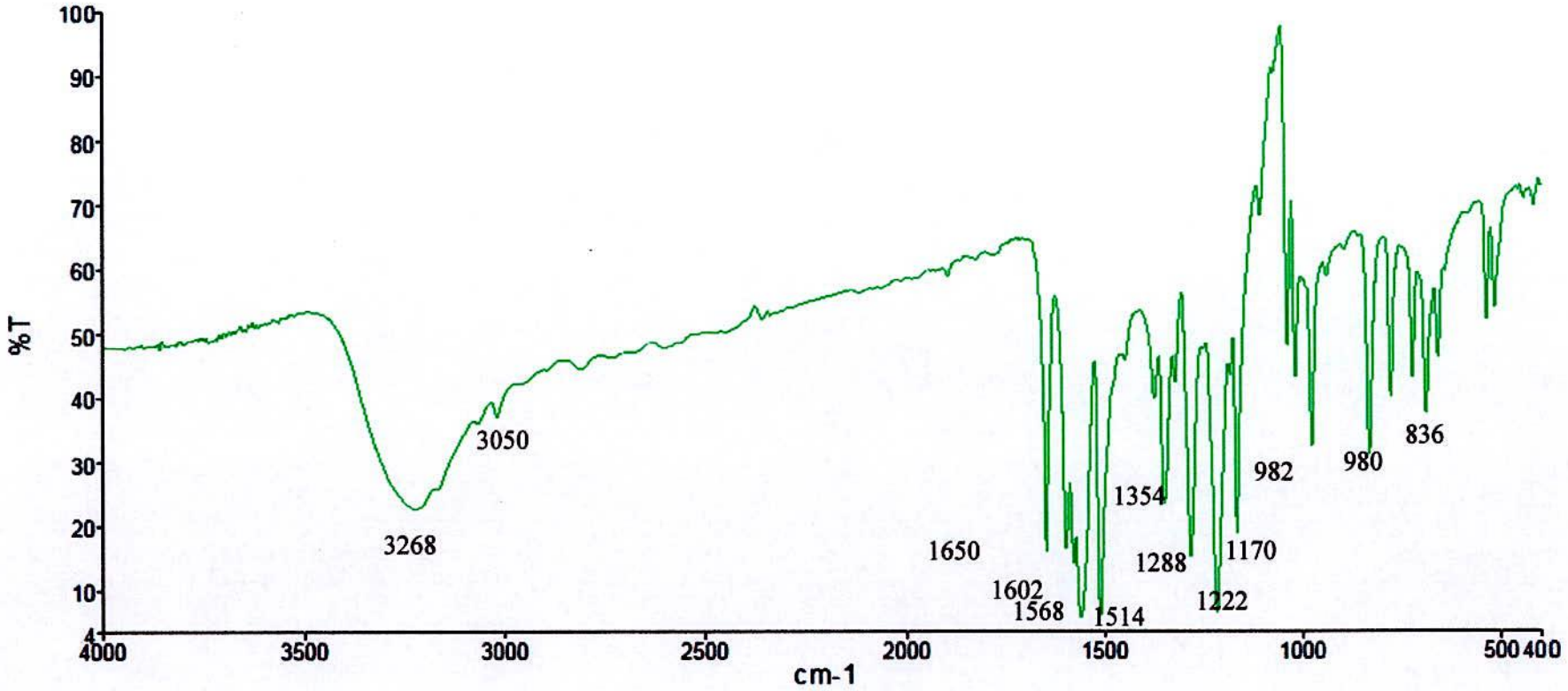


Fig 4.1: IR spectrum of the compound (I) as KBr pellets

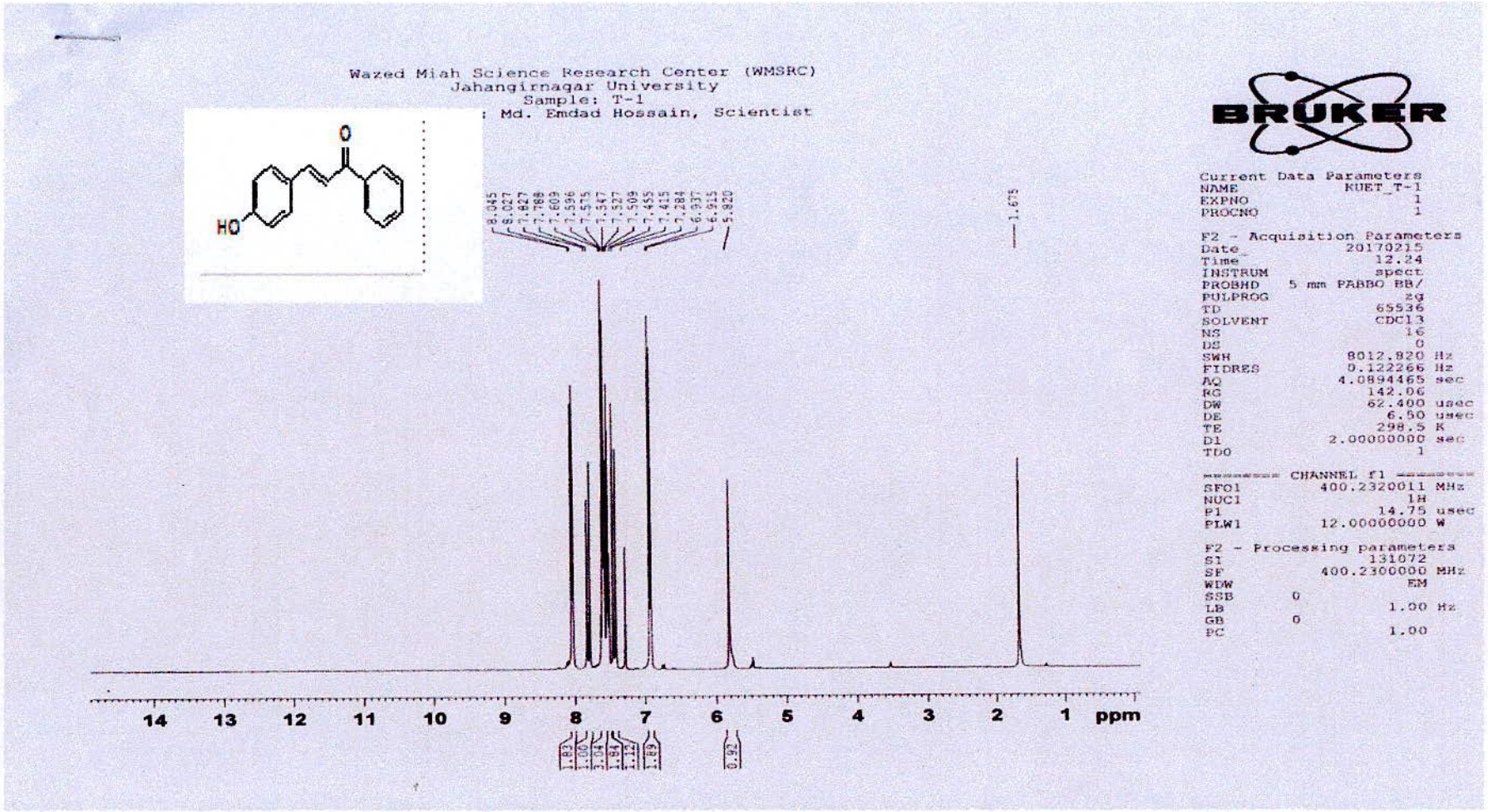


Figure 4.2: ^{1}H NMR spectrum of the compound (I) in $CDCl_3$

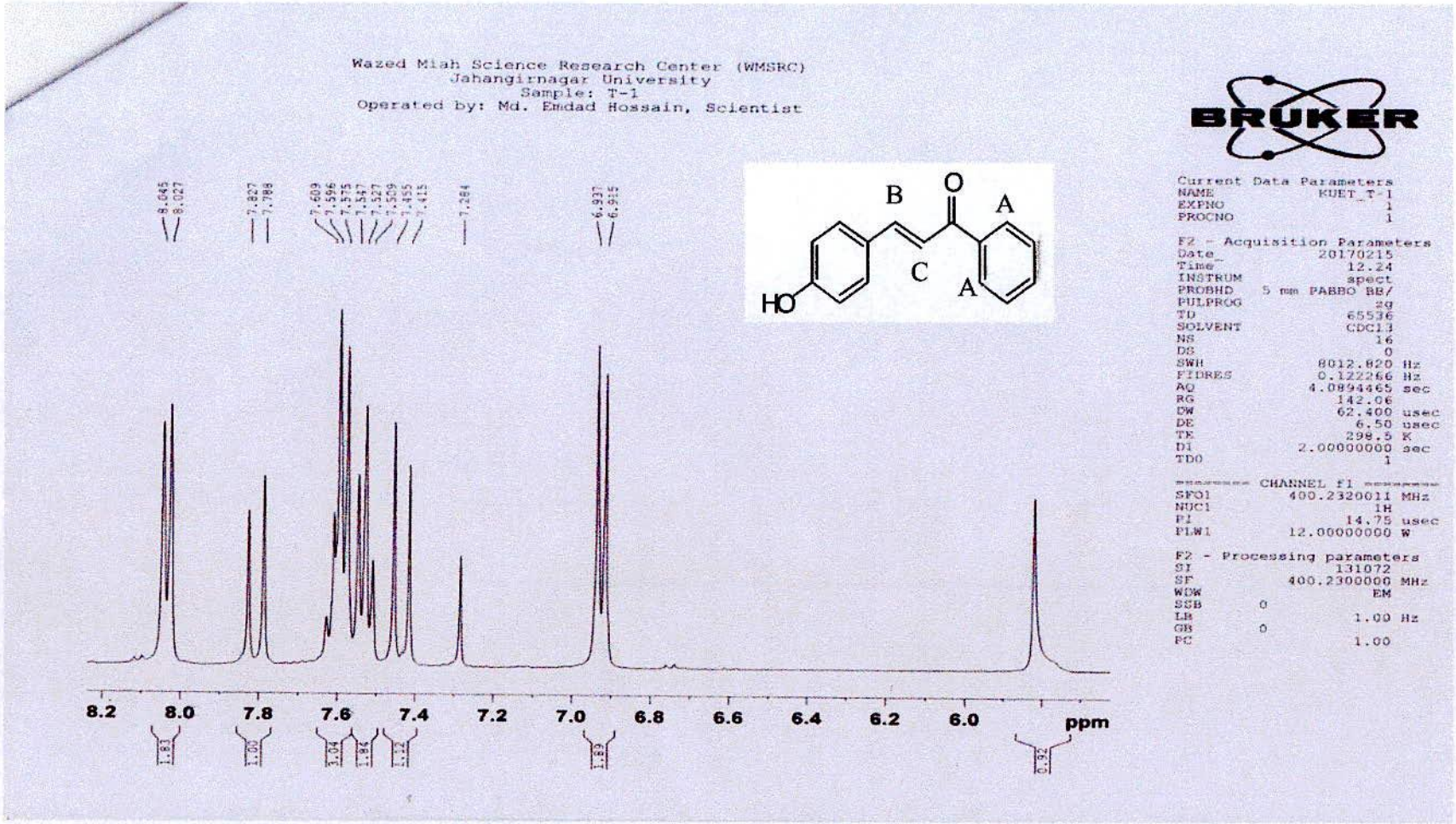
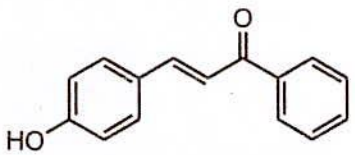


Fig 4 2: NMR spectrum of the compound (I) in $CDCl_3$

Comparison of spectral data of **1** with those published [100] permitted the identification of **1** as benzylideneacetophenone or *trans*-chalcone (IUPAC name: (E)-1,3-diphenylprop-2-en-1-one).The ^{1}H NMR and IR spectra of **1** which are comparable in chemical shift values and shape with the published spectra [100]. Protons A are the more deshielded due to the carbonyl's anisotropy cone. The *E* alkene presents two protons (B and C) with a wide common *J* value of 15.5 Hz, proton B is the more deshielded as consequence of its β position respect to carbonyl. The rest of protons present non-analyzable signals corresponding to7 aromatic protons distributed in both benzene rings

On the basis of the spectral properties (NMR, IR) and chemical behavior, structure (I) has been assigned to the obtain product.



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

(I)

4.2 Reaction of 3-(4-Hydroxy-phenyl)-1-phenyl-propenone with 2, 4-dinitro phenyl hydrazine.

The reaction of 3-(4-Hydroxy-phenyl)-1-phenyl-propenone and 2, 4-dinitro phenyl hydrazine give a white crystalline solid, m.p.230-231°C. The R_f value of the product was found to be 0.65. On [eluting solvent; $CHCl_3$=2:3].

Spectral properties

The ^{1}H NMR spectrum (Fig 4.3) of the compound in DMSO gave the following signals (***δ*** value) using TMS as an internal standard.

^{1}H NMR (400Mz $CDCl_3$) 8.342-8.371 (m, 1H, Ar), 8.126 (d, *J*=9.2 Hz, 1H, Ar), 8.033 (d, *J* = 7.2 Hz, 2H, Ar), 7.799 (d, *J* = 15.6 Hz, 1H), 7.351-7.686 (m, 7H, Ar), 7.178 (d, *J*= 16.4, 1H, NH), 6.835-6.986 (m, 2H), 6.517 (d, *J*= 16.4, 1H), 5.476 (s, 1H, OH).

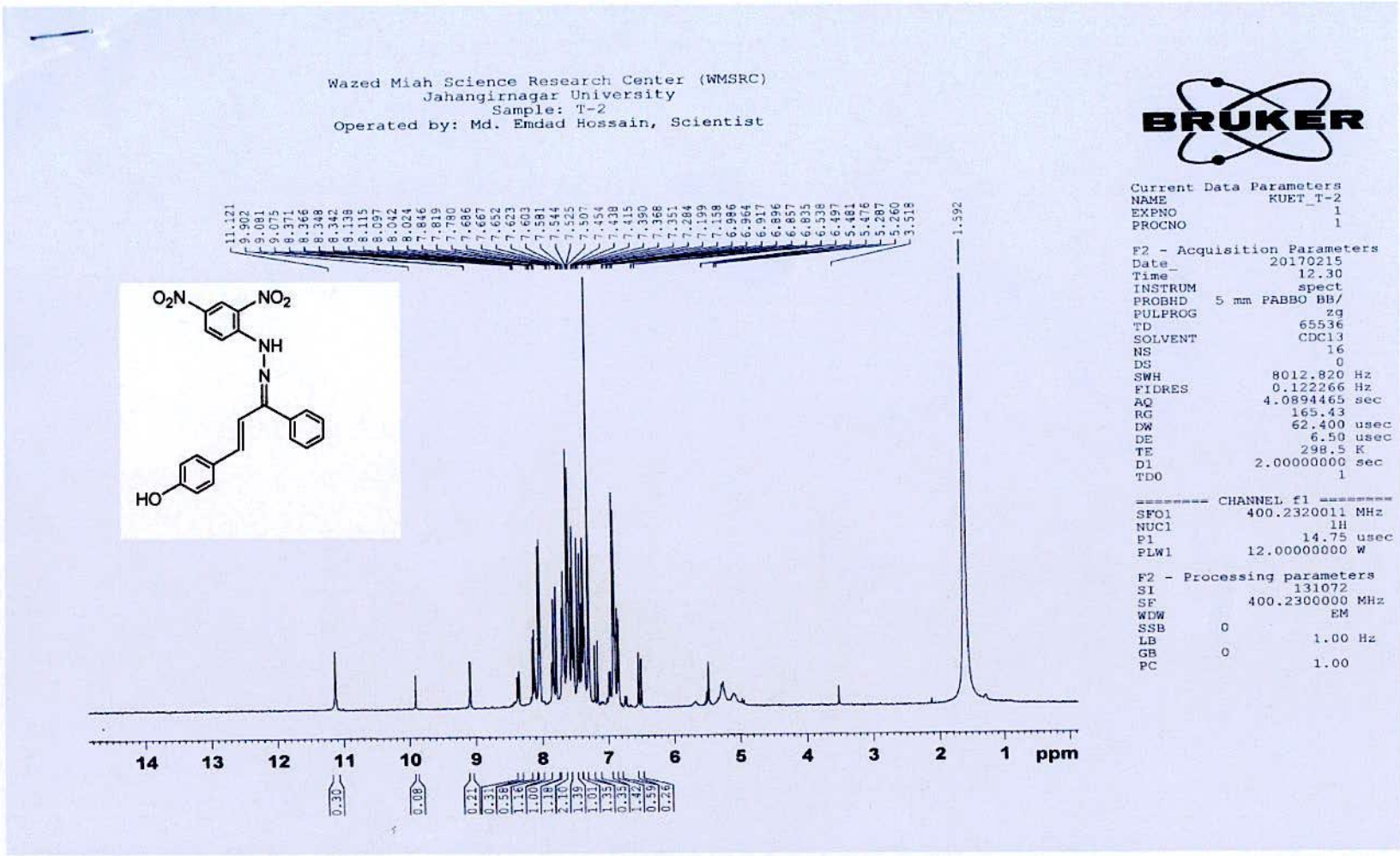


Fig 4.3: NMR spectrum of the compound (II) in $CDCl_3$

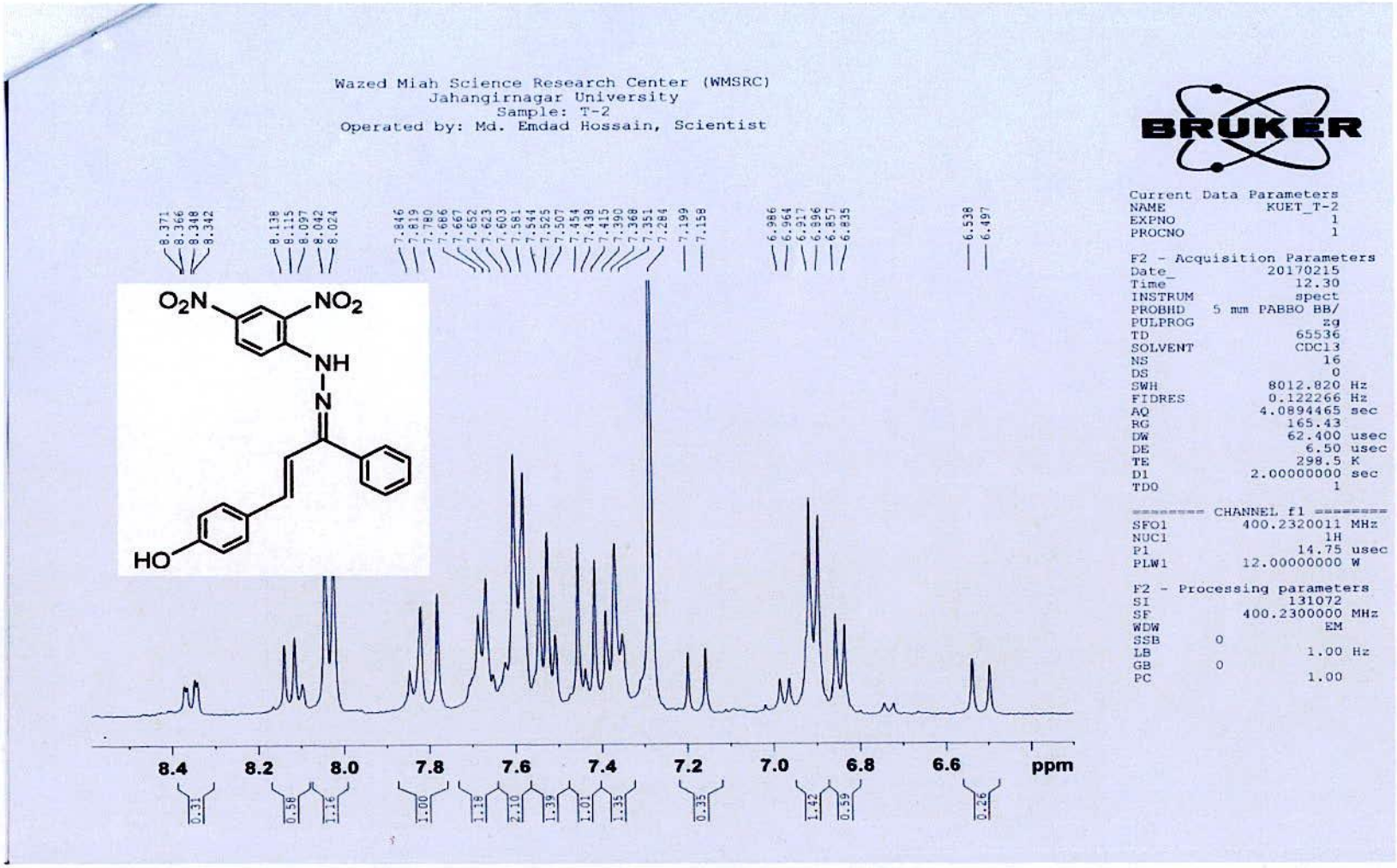
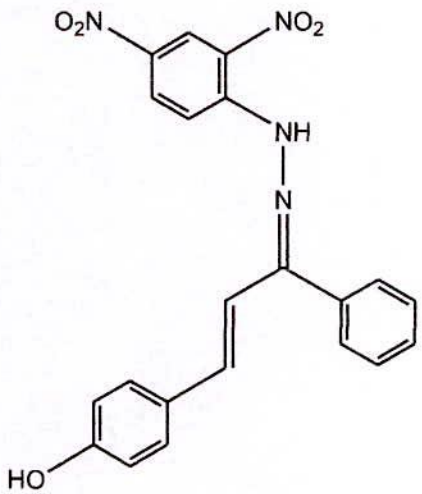


Fig 4.3 : NMR spectrum of the compound (II) in $CDCl_3$

On the basis of the spectral properties (NMR, IR) and chemical behavior, structure (II) has been assigned to the obtain product.



4-{3-[(2,4-Dinitro-phenyl)-hydrazono]-3-phenyl-propenyl}-phenol

(II)

4.3 Reaction of 3-(4-Hydroxy-phenyl)-1-phenyl-propenone with Dimedone Catalyst by anhydrous Zinc chloride

The reaction of 3-(4-Hydroxy-phenyl)-1-phenyl-propenone with dimedone in presence of anhydrous $Zncl_2$ in benzene at refluxing temperature gave a white crystalline solid, m.p.160-165°C. The R_f value of the product was found to be 0.76. On [eluting solvent; ethylacetate: n-hexane= 2:3].

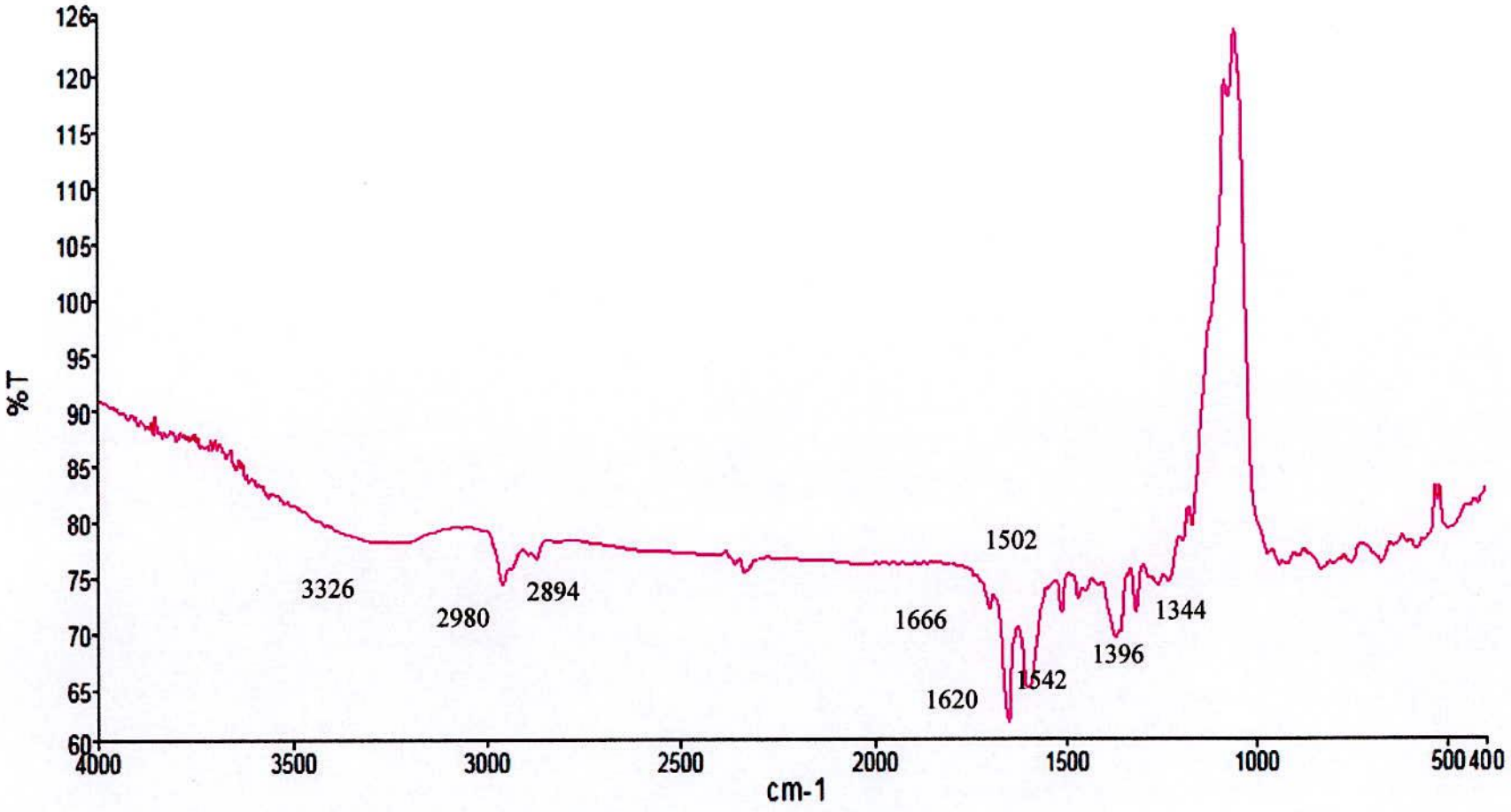


Fig 4.4: IR spectrum of the compound (III) as KBr pellets

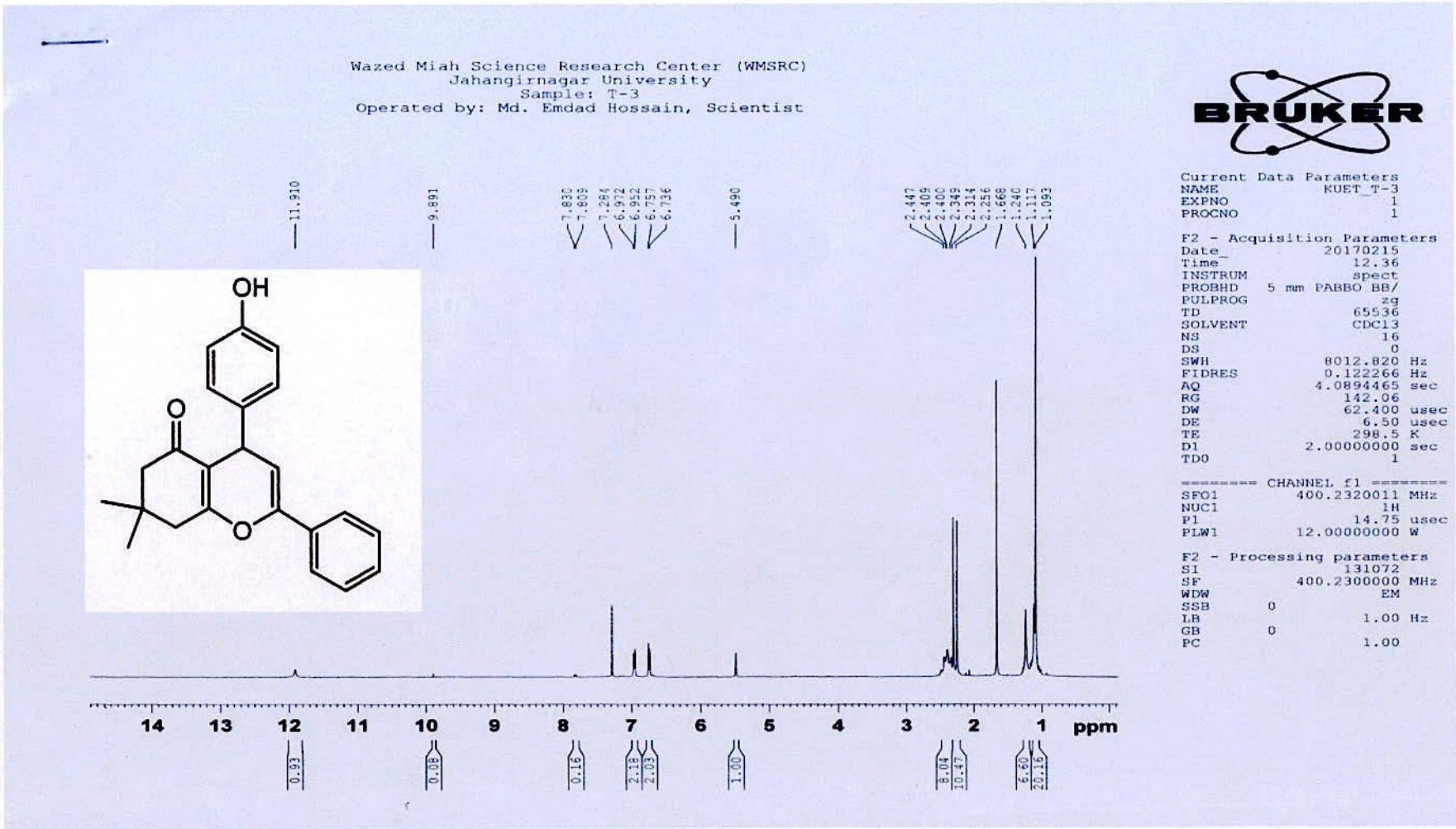


Fig 4.5: NMR spectrum of the compound (III) in $CDCl_3$

Spectral propertie

The IR spectrum (Fig 4.4) of the product run as KBr pellet showed the following stretching bands, V_{max} in cm^{-1}

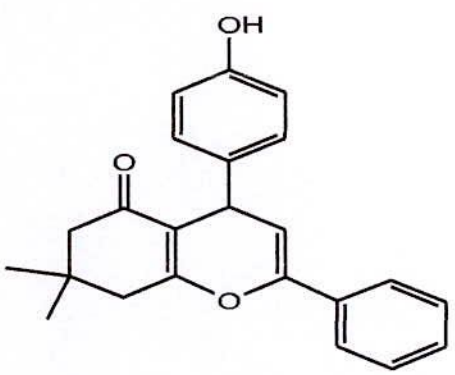
Table 4.2: IR value of compound III

3326	:OH group (H-bonding, intermolecular)
2980	:C-H aromatic
2894	:C-H aliphatic
1666	=C=O in conjugation
1620	=C=C in conjugation

The ^{1}HNMR spectrum (Fig 4.5) of the compound in DMSO gave the following signals (δ value) using TMS as an internal standard.

^{1}HNMR (400Mz $CDCl_3$) 11.910 (s, 1H, OH), 6.636-7.286 (m, 9H, Ar), 5.490 (s,2H), 2.314 (s, 2H, CH_2), 2.256 (s, 2H CH_2), 1.093 (s, 6H, CH_3).

On the basis of the spectral properties (NMR, IR) and chemical behavior, structure (III) has been assigned to the obtain product



(III)

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

4.4 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. Inhibition zone of some selected active compounds are given below:

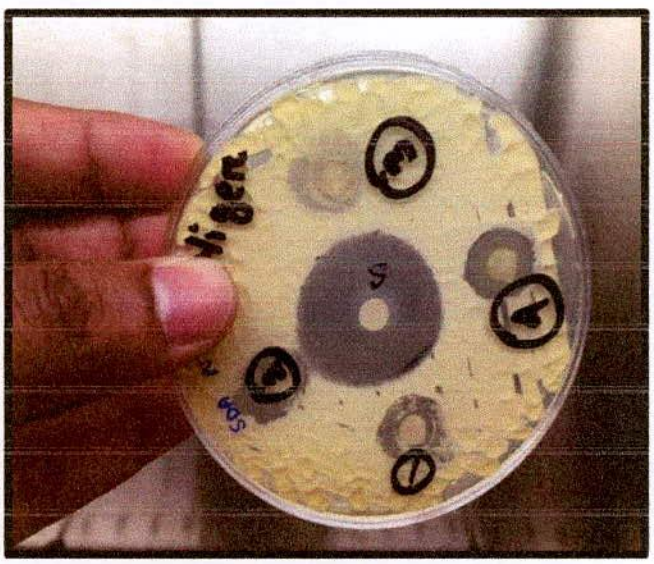


Figure 4.6: Antimicrobial activity of compounds 1,2,3 against *Staphylococcus aureus*

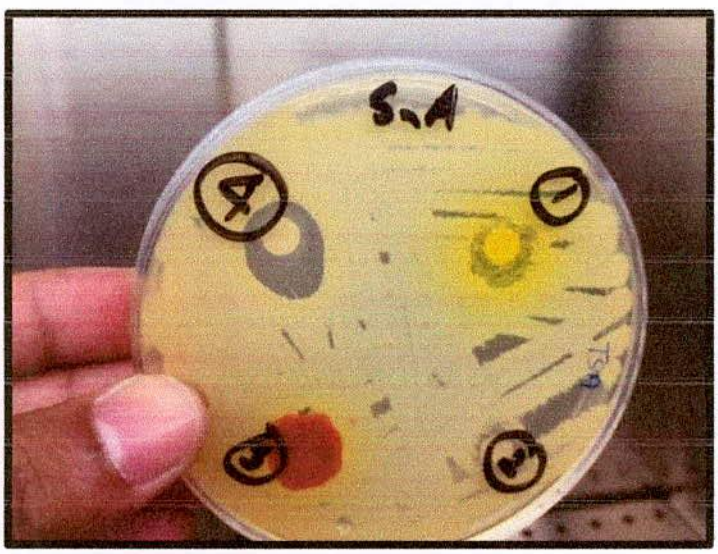


Fig 4.7: Antibacterial activity of compounds 1, 2, 3 against *Citobacter freund*

4.5 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 ciprofloxacin were recorded in mm and compared. Inhibition zone of some selected active compounds are given below

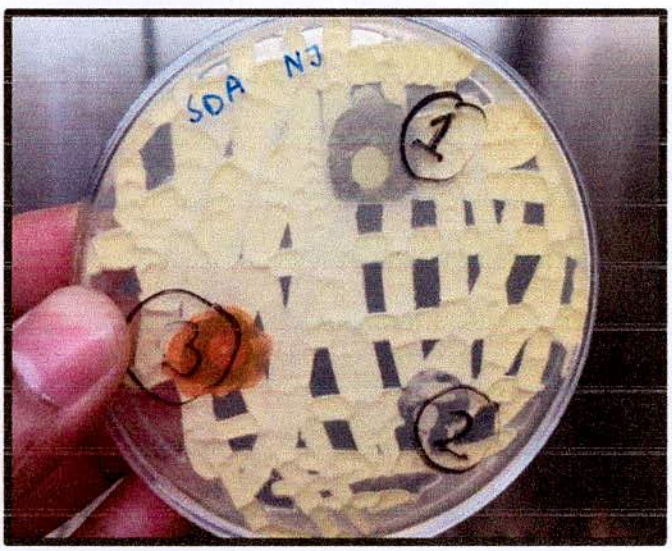


Figure 4.8: Antifungal activity of compounds 1, 2, 3 against *Tricodarma harzianum*

B.C. = *Bacillus cereus*

S.A. = *Staphylococcus aureus*

L.M = *Listeria monocytogenes*

E.C. = *Escherichia coli*

S.T. = *Salmonella typhimurium*

C.F. = *Citrobacter freundii*

T.H. = *Trichoderma harzianum*

Table 4.3 : Antibacterial and Antifungal activity of the synthesized compounds

Tasted sample	Name of Bacteria						Name of funghi
	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	30

Compound 1= (4-hydroxy phenyl)- 1-phenyl-propenone

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 7=1-(4-Amino-phenyl)-3-phenyl-propene

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 01, 02 and 03 with standard has given below:

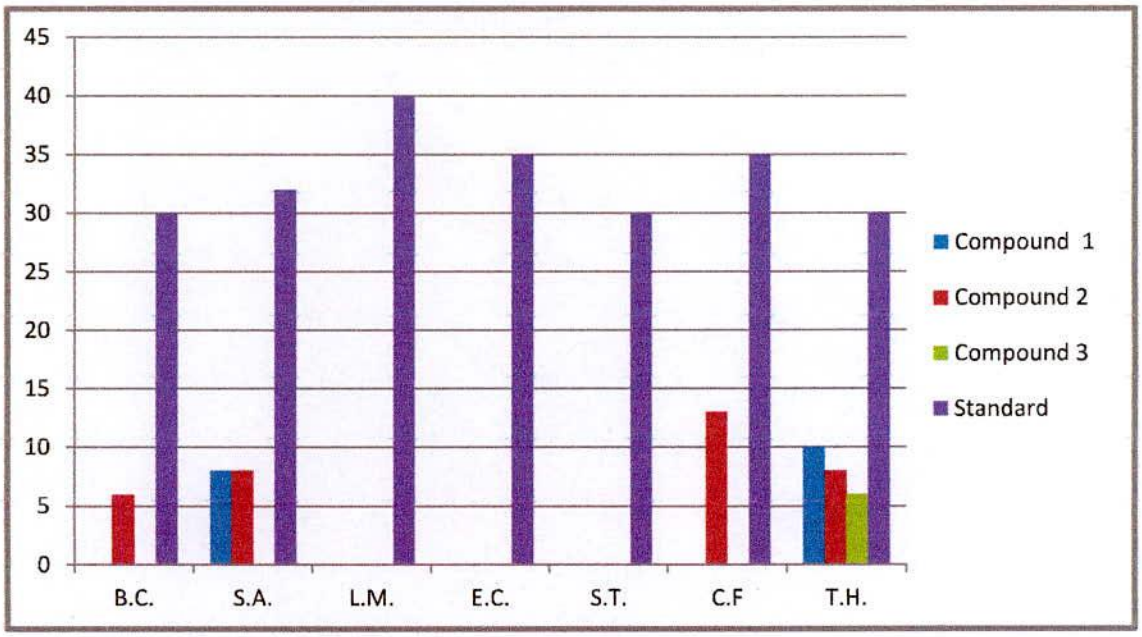


Fig 4.9: Zone of inhibition vs bacterial and fungal strain.

From the antimicrobial screening it has been observed that the synthesized compound 1 possessed no activity against the tested bacterial strains except S.A. having 8 mm zone of inhibition. Synthesized compound 2 exhibited moderate activity against two gram positive bacterial strains B.C. and S.A and one gram negative strain C.F. having inhibition zone 6, 8 and 13 mm respectively. Compound 3 did not show any antibacterial activity against the tested strains. All of the synthesized compounds produce moderate activity against the fungi strain T.H. compared to the standard used Miconazole. Compound 1, 2 and 3 showed zone of inhibition 10, 8 and 6 mm respectively against T.H. From the observed result it can be assumed that compound 2 (4-(4-Hydroxy-phenyl)-7,7-dimethyl-2-phenyl-4,6,7,8-tetrahydro-chromen-5-one) which was synthesized from compound 1 (4-{3-[(1-Methylene-4-nitro-but-2-enyl)-hydrazono]-3-phenyl-propenyl}-phenol) showed enhanced antibacterial activities than compound 1 and antibacterial activities diminishes at compound 3 (4-{3-[(1-Methylene-4-nitro-but-2-enyl)-hydrazono]-3-phenyl-propenyl}-phenol) which was synthesized from compound 2. Reverse order of activity was observed in case of the tested fungi T.H. The presence of unsaturated ketone function in synthesized compound may be responsible for their antibacterial and antifungal activity.

CHAPTER V

CONCLUSION

&

RECOMMENDATIONS

CHEAPTER V

Conclusion and Recommendations

This work described the synthesis, spectral characterization, and screening of antimicrobial activity of chalcone and chromene derivatives. First the chalcone was synthesized by p-hydroxyacetophenone and benzaldehyde by Claisen smith reaction using NaOH as a catalyst and their structures were elucidated on the basis of IR, ^{1}H NMR. This chalcone showed antibacterial activity against *Staphylococcus aureus* and showed good antifungal activity against *Trichoderma harzianum*. It was also observed that the chalcone possessed no activity against *Bacillus cereus, Listeria monocytogenes, Escherichia coli, Salmonella typhimurium, Citrobacter freundii*. The compound, **II** 4-{3-[(1-Methylene-4-nitro-but-2-enyl)-hydrazono]-3-phenyl-propenyl}-phenol did not show any activity against *Bacillus cereus, Listeria monocytogenes, Escherichia coli, Salmonella typhimurium, Citrobacter freundi, Staphylococcus aureus*. But the compound showed good antifungal activity against *Trichoderma harzianum.*The compound, **III** was successfully synthesized from chalcone and dimedone using zinc chloride as a catalyst and their structures were elucidated on the basis of IR, ^{1}H NMR analysis. The compound also synthesized by Microwave irradiation using ethanol as a solvent. This compound showed highly antibacterial activity against Bacillus *cereus, Staphylococcus aureus and Citrobacter freundii* but no activity against *Listeria monocytogenes, Escherichia coli. Salmonella typhimurium*. The compound, **III** showed good antifungal activity against *Trichoderma harzianum.*

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