SYNTHESIS, CHARACTERISATION AND ANTIMICROBIAL ACTIVITY OF THIAZOLE BASED CHALCONES

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ABSTRACT:

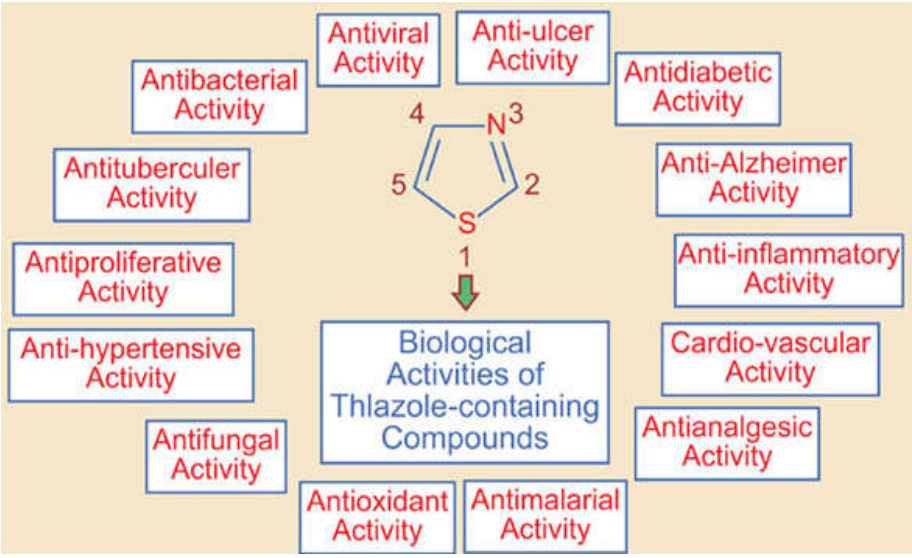
Chalcones are the important constituent of many natural sources and possess a variety of biological activities. A total six chalcone derivatives were synthesized by using different aromatic aldehydes. In this research work 5-acetyl-2,4-dimethylthiazole when condensed with equimolar proportions of aromatic aldehydes in the presence of 30 % alcoholic alkali at room temperature yield Chalcones. All synthesized compounds were purified by appropriate solvents identified and characterized by TLC, melting point, IR, NMR (^{1}H, ^{13}C), and mass spectroscopy. All 6 synthesized compounds were screened for antibacterial activity at concentrations 50 and 100 µg/ml. among all the compounds, 3c were found to have moderate active against gram –ve E. coli. Remaining all compounds were found to be having less or noactivity against gram +ve and gram –ve bacteria, compared to the standard streptomycin. for antifungal, concentrations 50 and 100µg/ml, out of which only 3c has shown a moderate activity against penicillium notatum compared with standard miconazole nitrate. Remaining all compounds was found to be inactive against fungi.

KEYWORDS: Chalcones, Thiazole,Anti-Fungal,Spectroscopy,Antibacterial,NMR.

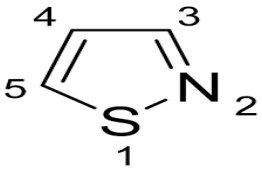
INTRODUCTION

1. Thiazole:

Thiazole, or 1, 3-thiazole, is a heterocyclic compound that contains both sulfur and nitrogen; the term 'thiazole' also refers to a large family of derivatives. Thiazole itself is a pale-yellow liquid with a pyridine-like odour and the molecular formula C_3H_3NS. The thiazole ring is notable as a component of the vitamin thiamine (B_1)



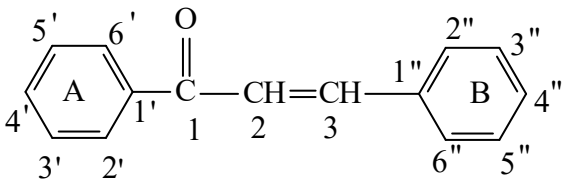
Structure:



1.2 CHALCONE AND ITS DERIVATIVES:

Chalcone is a generic term given to compounds bearing the 1, 3-diphenyl-2-propen-1-one framework and belong to the flavonoid family[1-3]. Chemically they are open-chain flavonoids in which the two aromatic rings are joined by a three carbon α, β-unsaturated carbonyl system. Chalcones are abundantly present in nature starting from ferns to higher plants[4] and a number of them are polyhydroxylated in the aryl rings. In plants, chalcones are converted to the corresponding (2S)-flavanones in a stereospecific reaction catalyzed by the enzyme chalcone isomerase. This close structural and biogenetic relationship between chalcones and flavanones explains why they often co-occur as natural products.

General structure of chalcone*:*



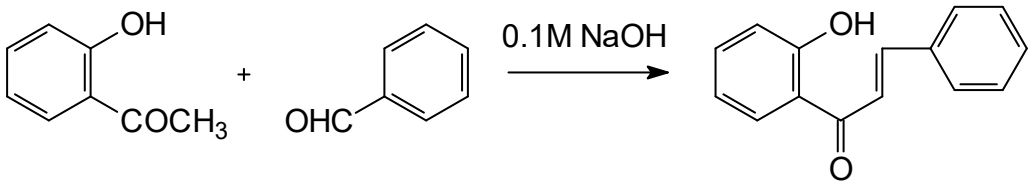
All the chalcones give pink coloration with concentrated sulphuric acid in Wilson's test[5] and when a phenolic hydroxyl group is present, they give violet coloration with alcoholic ferric chloride solution.

Chalcones on heating with traces of iodine in dimethyl sulphoxide (DMSO) for two hours give the corresponding flavones. Chalcones were converted into the corresponding flavanol's by their oxidation using hydrogen peroxide in methanolic sodium hydroxide solution and these flavanol's showed a characteristic greenish yellow fluorescence in ethanolic solution as well as with concentrated sulphuric acid.

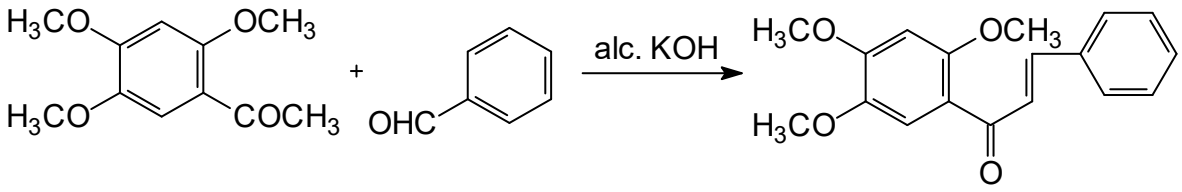
GENERAL METHODS OF SYNTHESIS OF CHALCONES

Chalcones can be obtained by the acid or base catalysed aldol condensation of acetophenones with aromatic aldehydes.

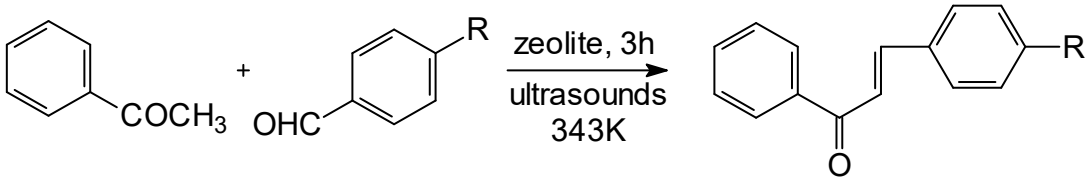
1. 2'-hydroxyacetophenone react with benzaldehyde in the presence of 0.1M NaOH to give the chalcone



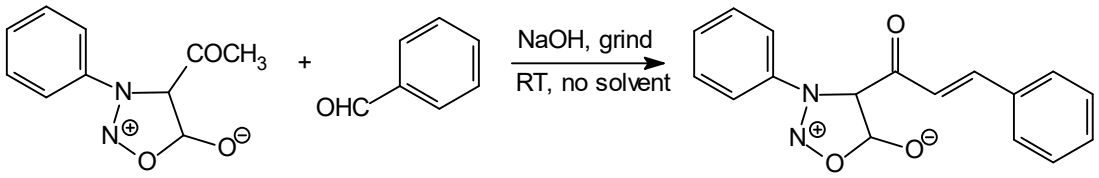
2. 2',4',5'-trimethoxyacetophenone, when condensed with equimolar proportions of aromatic aldehydes in the presence of 30 % alcoholic alkali at room temperature yield chalcones



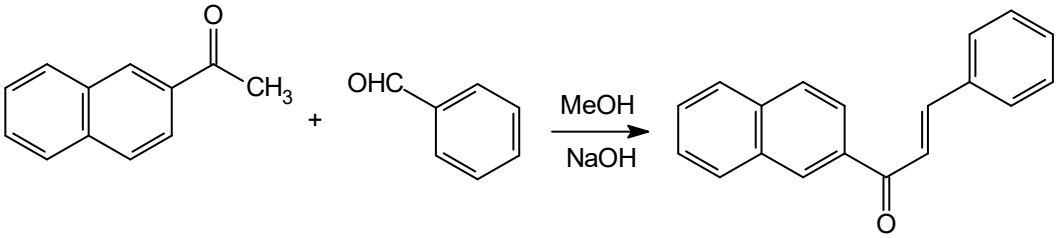
3. Claisen-Schmidt condensation between benzaldehyde and acetophenone by chemical and thermally activated reactions over zeolite as catalyst under solvent free conditions give chalcone.



4. 4-acetyl-3-aryl-syndones when subjected to grinding with various aryl aldehydes in the presence of a base catalyst under solvent free conditions yield syndone chalcones .

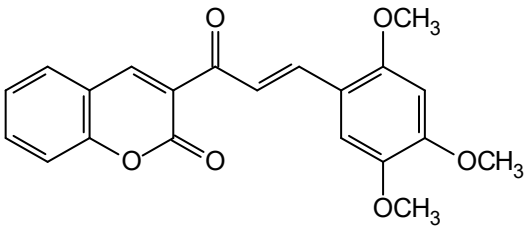


6. Condensation of 2-naphthylmethyl ketones with substituted aryl aldehydes in the presence of NaOH under methanol as solvent gave the corresponding chalcones.

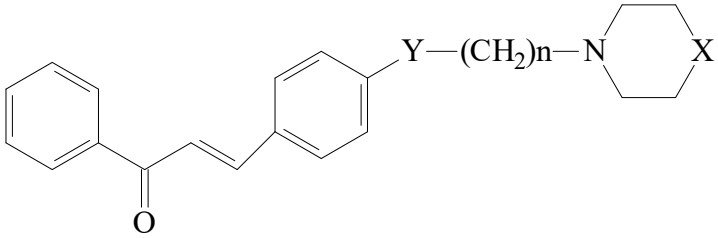


LITERATURE REVIEW:

Synthesized 3-[1-oxo-3-(2,4,5-trimethoxyphenyl)-2-propenyl]-2H-1-benzopyran-2-ones that showed significant antimicrobial activity against B.subtilis, B.pumilis and E.coli when tested at a concentration of 1000 µg/ml. The study revealed the importance of electron releasing groups such as hydroxyl and methoxyl groups in enhancing the activity. Chalcones with halogen substituents like bromine and chlorine contributed favorably to the antifungal activity.



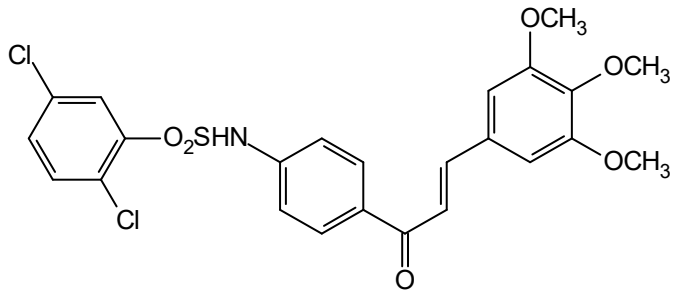
synthesized a series of substituted chalcones and tested for their antibacterial and antifungal activities. The physico-chemical properties of these novel chalcones which contributed favorably to the observed activities were also determined.



Y=S, O; n=4, 5, 6; X=CH-CH_3, O, NH

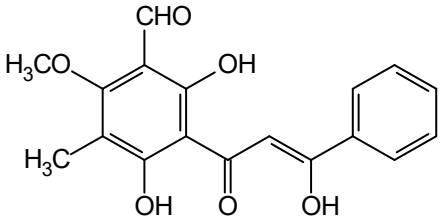
Antimalarial activity:

synthesized chalcones with sulfonamide moiety possessing antimalarial activity.



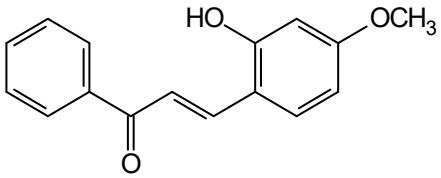
Anti-HIV activity:

isolated a chalcone that exhibited the anti-HIV activity with a good therapeutic index.



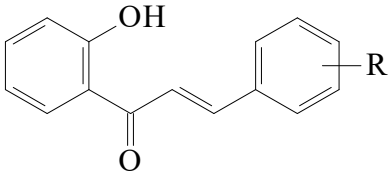
Antitubercular activity:

synthesized a chalcone that exhibited antitubercular activity.



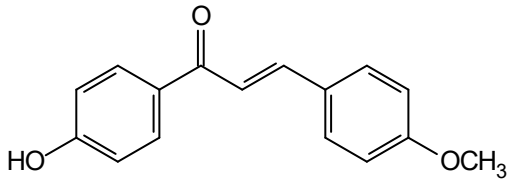
Antioxidant activity:

synthesized 2'-hydroxychalcones which showed antioxidant activity.



Miscellaneous activities:

synthesized a 4'-hydroxy-4-methoxychalcone exhibiting antihyperglycemic activity.



AIM AND OBJECTIVES

1. It is proved from the literature that apart from possessing several biological activities, chalcones are also useful intermediates for the synthesis of several chemical and pharmacological classes of therapeutic agents having heterocyclic structures in them. We synthesize some new substituted chalcones by Claisen-Schmidt condensation reaction in the present study.
2. To synthesize thiazole based new chalcones of 5-acetyl-2, 4-dimethylthiazole by reaction with various aromatic aldehydes.
3. To characterize the synthesized chalcones using IR, ^{1}H NMR, and Elemental analysis data. The data related to structural characterization are given individually.

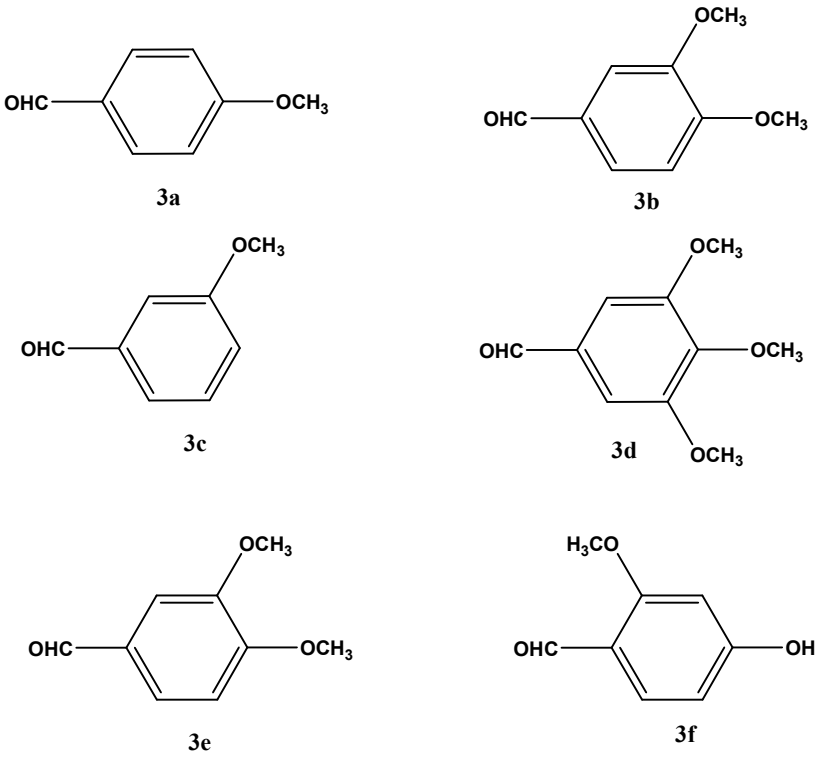
SCHEME OF THE SYNTHESIS

General procedure for the synthesis of chalcones by Claisen-Schmidt condensation Synthesis of the thiazolyl chalcones (3a-3f):

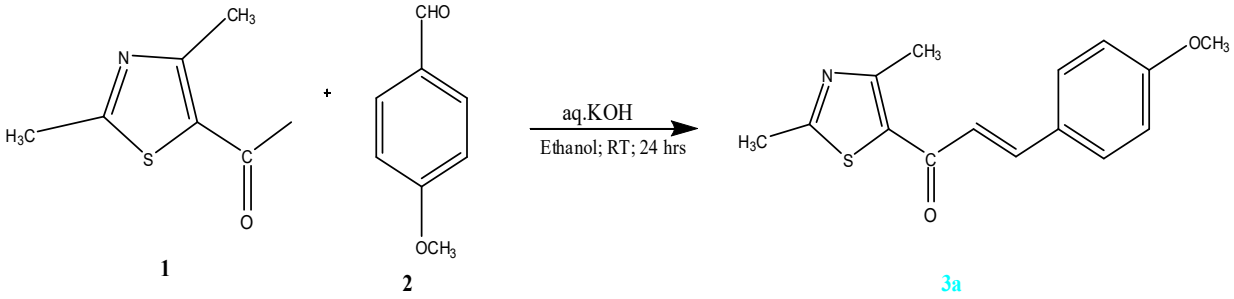
Equimolar quantities (0.005mol) of 5-acetyl-2,4-dimethylthiazole and respective aldehydes were mixed and dissolved in 4 ml of alcohol. To this, aqueous potassium hydroxide solution (50%, 7.5 ml) was added slowly and mixed occasionally for 24 hours, at room temperature. Completion of the reaction was identified by TLC using silica gel-G. After completion of the reaction, the mixture was poured onto crushed ice, acidified, if necessary, with dilute hydrochloric acid, and the solid that separated was isolated by filtration, dried and purified by recrystallization using methanol or chloroform as the solvents.

Scheme: Synthesis of thiazolyl chalcones

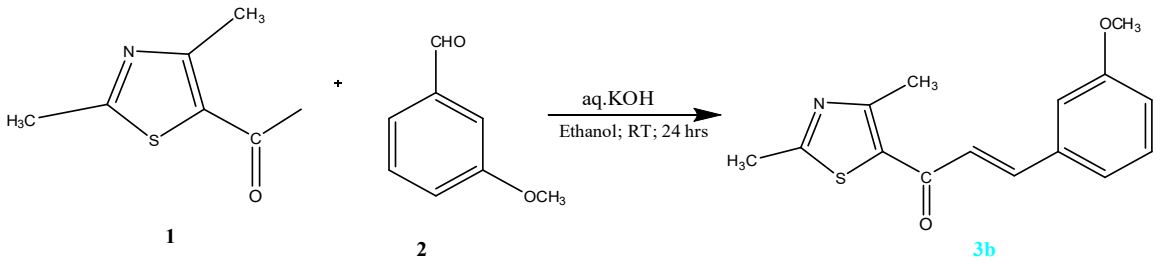
Different Aldehydes (3a-3f) Used



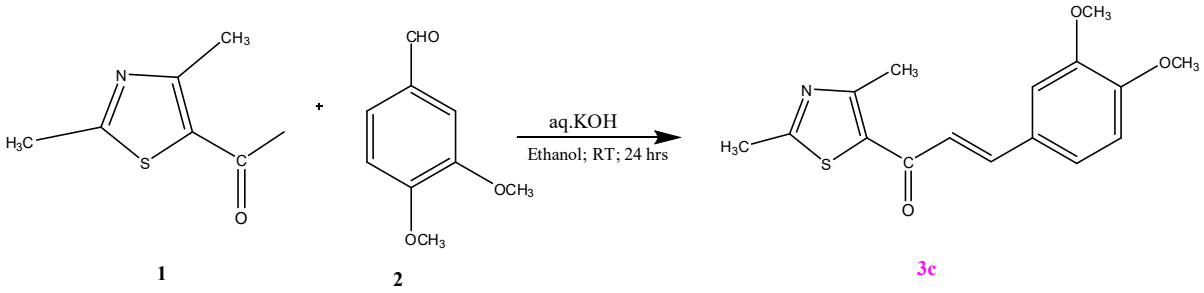
5-acetyl-2,4-dimethylthiazole when condensed with equimolar proportions of aromatic aldehydes in the presence of 30 % alcoholic alkali at room temperature yield chalcones (Scheme 1).



5-acetyl-2,4-dimethylthiazole when condensed with equimolar proportions of aromatic aldehydes in the presence of 30 % alcoholic alkali at room temperature yield chalcones (Scheme 2).

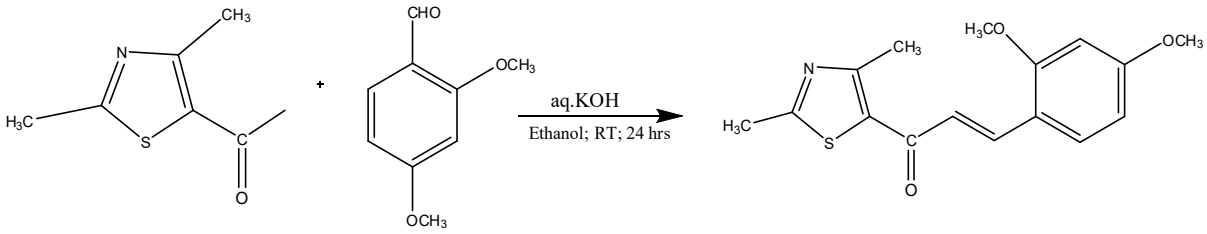


5-acetyl-2,4-dimethylthiazole when condensed with equimolar proportions of aromatic aldehydes in the presence of 30 % alcoholic alkali at room temperature yield chalcones[11] (**Scheme 3**).



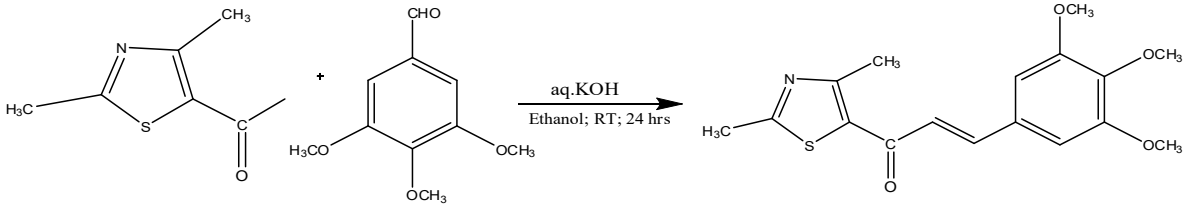
1 2 3c

5-acetyl-2,4-dimethylthiazole when condensed with equimolar proportions of aromatic aldehydes in the presence of 30 % alcoholic alkali at room temperature yield chalcones[11] (**Scheme 4**)



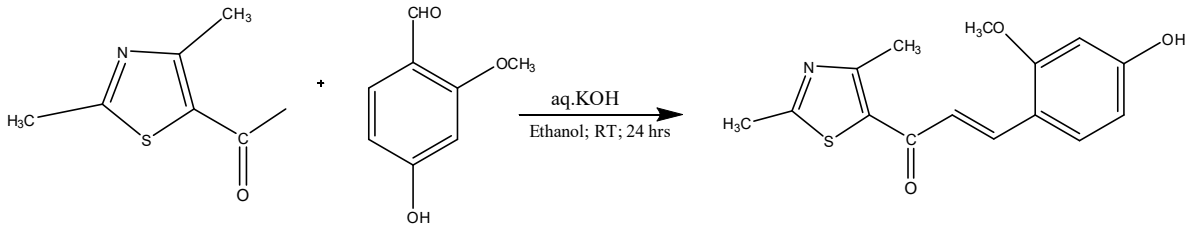
1 2 3d

5-acetyl-2,4-dimethylthiazole when condensed with equimolar proportions of aromatic aldehydes in the presence of 30 % alcoholic alkali at room temperature yield chalcones[11] (**Scheme 5**)



1 2 3e

5-acetyl-2,4-dimethylthiazole when condensed with equimolar proportions of aromatic aldehydes in the presence of 30 % alcoholic alkali at room temperature yield chalcones[11] (**Scheme 6**).



1 2 3f

4.4 IDENTIFICATION AND CHARACTERIZATION

The compounds synthesized were identified and characterized by following methods such as:

- Melting point determination
- Thin layer chromatography
- Infra-red spectroscopy
- Nuclear magnetic resonance spectroscopy (^{1}H NMR and ^{13}C NMR).
- Mass spectroscopy

MELTING POINT DETERMINATION:

The melting point of organic compound was determined by Thiel's melting point tube (capillary tube method). The determination of melting point is the most important and easy way of differentiating the physical constant of one compound from other.

THIN LAYER CHROMATOGRAPHY (TLC):

TLC is an important method for synthetic chemistry to the formation of the compound based on the Rf values since different compounds will have different Rf values. It also helps in confirming the progress of the reaction. The solvent used was ethanol: toluene: acetone.

INFRA RED SPECTROSCOPY (IR):

IR is one of the most important tools for determining the various functional groups and the possible chemical structure. The IR spectra of compounds were carried out in university college of pharmacy, Guntur.

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (NMR):

The ^{1}H NMR and ^{13}C NMR spectra of the compounds were carried out in Bruker AMX 400 MHz NMR at LAILA IMPLEX, Vijayawada. Particular solvents like deuterated chloroform, deuterated ethanol and deuterated DMSO were used for the particular derivative compounds.

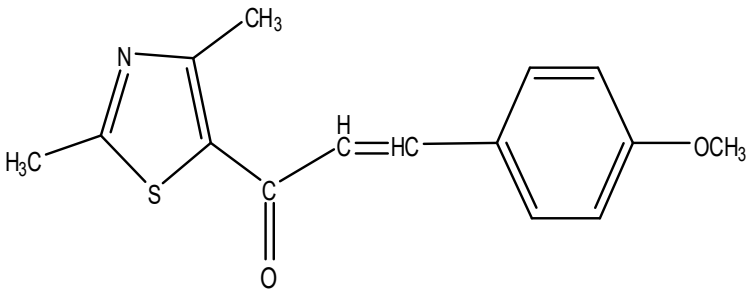
MASS SPECTROSCOPY:

It is used for elucidating the chemical structure of molecules; the MS principle consists of ionizing chemical compounds to generate charged molecules or molecule fragments and measurement of their mass to charge ratios. The compound spectra of the compound were carried out in Agilent 1100 series LC-MSD at LAILA IMPLEX, Vijayawada.

CHARACTERIZATION

Characterization of the synthesized compounds

(3a) 1-(2,4-dimethylthiazol-5-yl)-3-(4-methoxyphenyl)prop-2-en-1-one

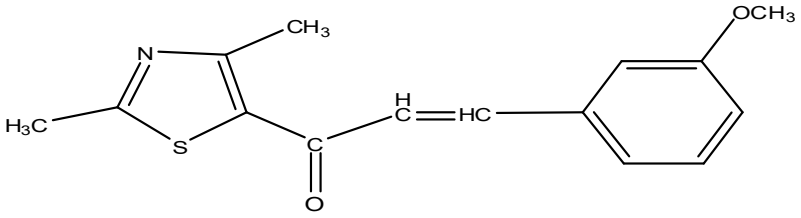


M.P : 203-205˚
Rf : 0.44

Molecular weight : 273.35 gr/mol
Molecular formula : C15H15N02
%Yield : 77%
Solubility : Chloroform

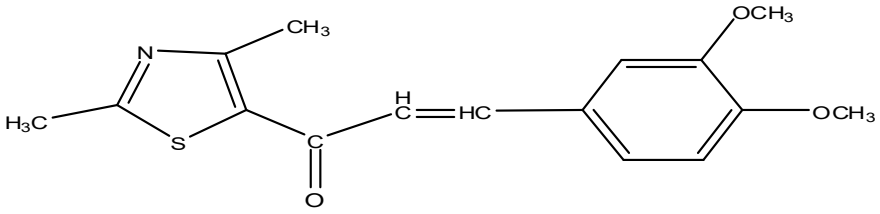
(3b)

1-(2,4-dimethylthiazol-5-yl)-3-(3-methoxyphenyl)prop-2-en-1-one



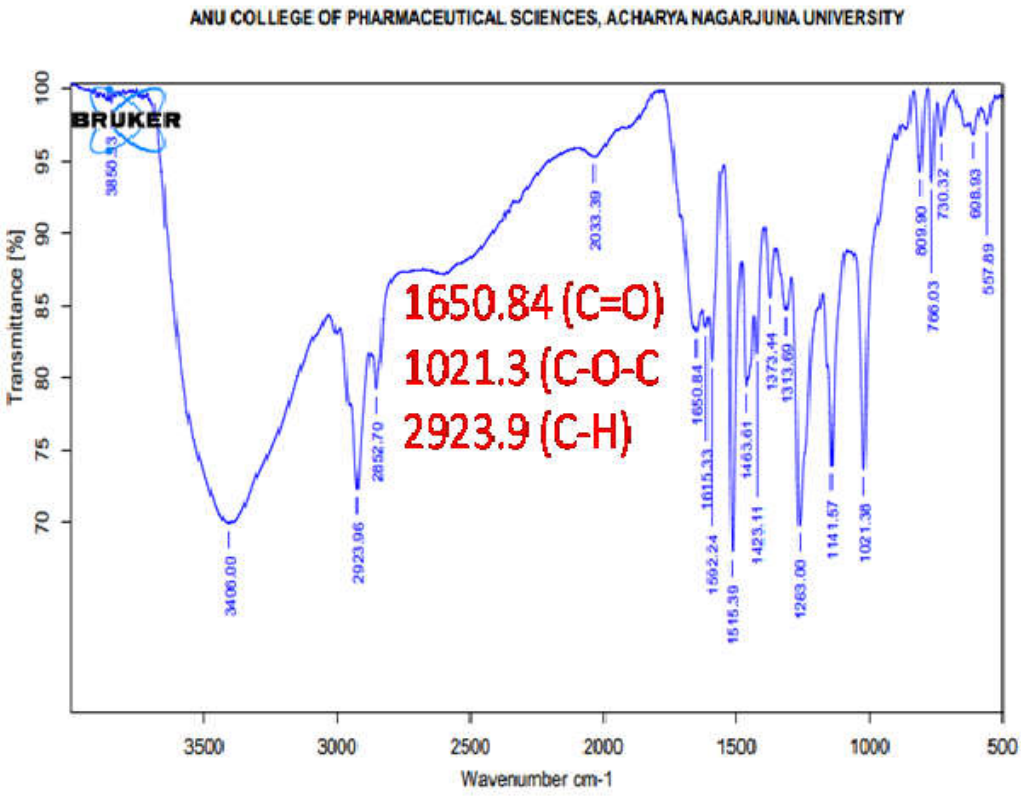
M.P. : 201-203
Rf : 0.44
Molecular weight : 273.35
Molecular formula : $C_{15}H_{15}N0_2$
%Yield : 76%
Solubility : chloroform

(3c) 3-(3,4-dimethoxyphenyl)-1-(2,4-dimethylthiazol-5-yl)prop-2-en-1-one



M.P : 164-166
Rf : 0.54
Molecular weight : 303.38
Molecular formula : $C_{16}H_{17}NO_3S$
%Yield : 87%
Solubility : ethanol

IR specrum of compound 3C


ANU COLLEGE OF PHARMACEUTICAL SCIENCES, ACHARYA NAGARJUNA UNIVERSITY
BRUKER
Transmittance [%]
Wavenumber cm-1
3500
3000
2500
2000
1500
1000
500
1650.84 (C=O)
1021.3 (C-O-C
2923.9 (C-H)
3400.00
2923.06
2852.70
2033.39
1650.84
1615.33
1592.24
1515.39
1463.61
1423.11
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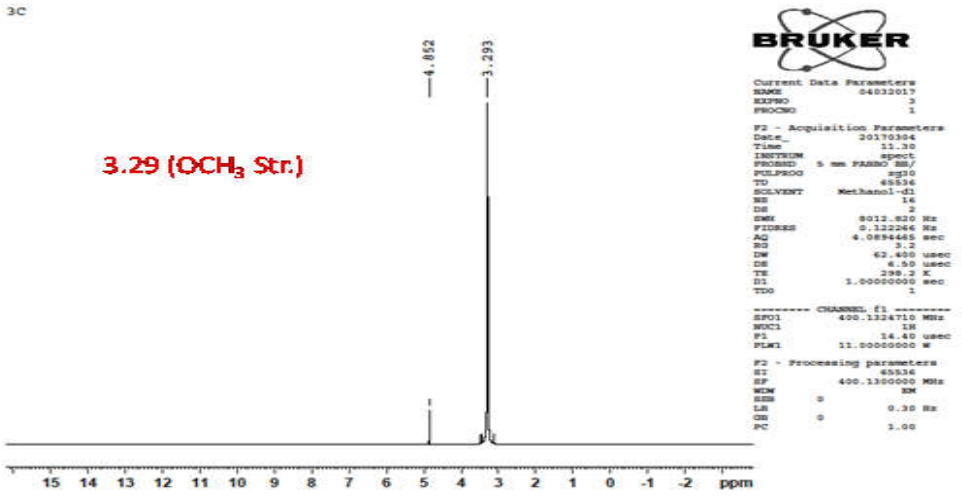
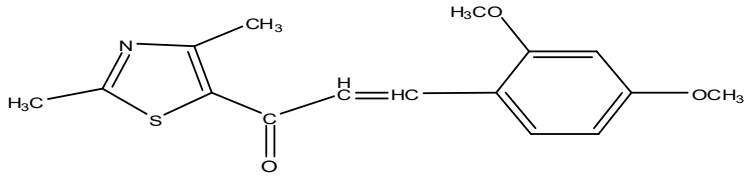
766.03
730.32
698.93
557.69


3C
3.29 (OCH3 Str.)
4.852
3.293
BRUKER
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 ppm

(3d)



3-(2,4-dimethoxyphenyl)-1-(2,4-dimethylthiazol-5-yl)prop-2-en-1-one

M.P : 162-164

Rf : 0.45

Molecular weight : 303.38

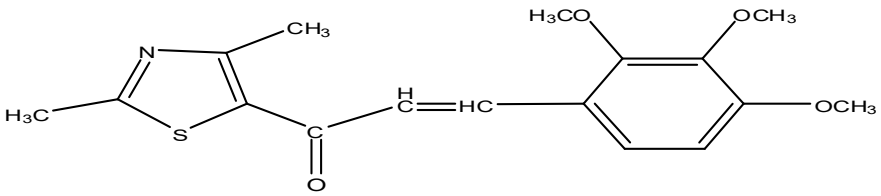
Molecular formula : $C_{16}H_{17}NO_3S$

%Yield : 86%

Solubility : chloroform

(3e)

1-(2,4-dimethylthiazol-5-yl)-3-(2,3,4-trimethoxyphenyl)prop-2-en-1-one



M.P : 208-210

Rf : 0.54

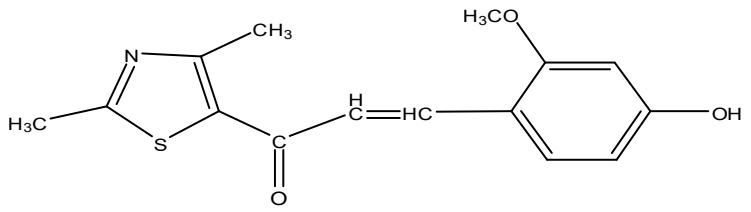
Molecular weight : 333.40

Molecular formula : $C_{17}H_{19}N0_4S$

%Yield : 84

3(F)

1-(2,4-dimethylthiazol-5-yl)-3-(4-hydroxy-2-methoxyphenyl)prop-2-en-1-one



M.P : 248-250

Rf	: 0.64
Molecular weight	: 289.35
Molecular formula	: $C_{15}H_{15}N0_3S$
%Yield	: 81%
Solubility	: ethanol

The synthesized derivatives of Chalones were subjected to examination of the following biological studies:

1) Anti-microbial

5.1 ANTI MICROBIAL ACTIVITY

5.1.1 ANTI BACTERIAL ACTIVITY

The antibiotic potency can be determined using the microbial assay, as convenient chemical methods are not suitable. It reflects the concentration and not the activity of the antibiotics.

The microbiological assay is based upon a comparison of the inhibition of microorganisms by measured concentrations of the antibiotics to be examined, with that produced by known concentration of a standard preparation of antibiotic having known activity two general methods are usually employed

1) Cylindrical or cup plate method.
2) Turbidimetric method or tube assay method

Antibacterial activity:

Method: Cup Plate method was used to carry out this study.

Principle:

The cup plate method depends on diffusion of antibiotic from a cup through a solidified agar layer in a petri dish or petri plates to an extent such that growth of added microorganism is prevented entirely in a zone around cup or cylindrical containing a solution of antibiotic,

A. Preparation of test solutions:

Synthesized compound of quinolinyl pyrazolines were dissolved in minimum amount of dimethyl sulfoxide (DMSO) and volume made with DMSO, to get 50 and 100 µg/ml concentrations.

B. Preparation of standard solutions:

Streptomycin was the reference standard drug prepared in water to get 50 µg/ml

C. Test organisms used were:

1. Gram –ve Escherichia coli.
2. Gram +ve Staphylococcus aureus
3. Gram +ve Bacillus pimilis.

D. Preparation of sub culturing media:

Peptone water media was prepared using following ingredients.

1. Beef extract – 10g
2. Peptone – 10g
3. Sodium chloride – 5g
4. Distilled water – Q.S. to 1000ml

E. Preparation of media:

1. Peptone – 6g
2. Casein hydrolysed of soyabean – 4g
3. Yeast extract – 3g
4. Beef extract – 1.5g
5. Dextrose (dehydrated) – 1g
6. Agar – 15g
7. Distilled water sufficient to make 1000ml

The above shown quantities of different ingredients were accurately weighed and dissolved in 1litre of distilled water. The media was distributed equally in to 4 conical flasks, then sterilized by autoclaving at 15 Lbs/Sq. inch for 15 minutes.

F. Preparation of inoculum:

The peptone water medium was sterilized by autoclaving at 15Lbs/sq. inch for 15min. loop full organisms were transferred from a laboratory-maintained culture in to a conical flask (250 ml) containing sterilized peptone water medium. The flask was incubated for 24hrs at 37°c.

G. Sterilization of apparatus required:

Petri dishes, cork borer (8mm), glass syringes and test tubes were sterilized by autoclaving at 15Lbs/sq. inch for 15min.

I. Procedure for microbial assay:

Each conical flask with the medium was cooled to 46°C and inoculated with test organism (20ml of subculture medium per 100ml of the assay medium). To 20ml each of inoculated media was distributed into petri plates and maintained at room temperature (each reading was taken in triplicate). When media was solidified, four cups (8mm diameter) were made using sterile cork borer. Two drops of each of the test solutions as well as standard solutions and blank (DMSO) were placed in each cups separately under septic condition, the petri plates were kept in the refrigerates for 2hrs to allow the uniform diffusion of drug into the agar medium.

All the petri plates were then incubated at 37°c for24hrs and zones of inhibition (in mm) were measured and shown in the Table no's 6.1, 6.2 and 6.3.

5.1.2 ANTI FUNGAL ACTIVITY

Anti-fungal activity:

The cup plate technique described by Hugo and pussel (1984) was employed in studying the anti-fungal activity.

Principle:

The cup plate method depends on diffusion of antibiotic from a cup through a solidified agar layer in a petri plate to an extent such than growth of added microorganism is prevented entirely in a zone around cup or cylinder containing a solution of antibiotic.

A. Preparation of standard solutions:

Miconazole nitrate was the reference standard drug prepared in DMSO to get 50 µg/ml and 100 µg/ml.

B. Test organisms used were:

1) Penicillium notatum
2) Aspergillus Niger

C. Preparation of sub culturing media:

1) Beef extract – 3.0g
2) Peptone – 5.0g
3) Sodium chloride – 5.0g
4) Distilled water – Q.S. to 1000ml

D. Preparation of culture medium:

1) Peeled potato – 200-300gr
2) Dextrose – 5gr
3) Agar – 20g
4) Distilled water – q,s 1000ml

E. Preparation of inoculums:

The subculture media was sterilized by autoclaving at 15Lbs/sq. inch for 15min. a loop full of organisms was transferred from a laboratory-maintained mother culture in to a 25ml conical flask containing sterilized subculture medium. The flask was incubated for 48hrs at 37°c.

F. Sterilization of apparatus:

Petri dishes, glass syringe, cork borer (8mm), conical flask and test tubes were sterilized by autoclaving at 15Lbs/sq. inch for 15min.

G. Procedure for microbial assay:

Each conical flask with the medium was cooled to 46°C and inoculated with test organism (20ml of subculture medium /100ml of the assay medium). Then 20ml of inoculated media was distributed into petri dishes. After solidification of the media, four bores were made at equal distance by using a sterile cork borer (8mm diameter). A single concentration (50 µg/ml) of standard drug and different concentrations (50 and 100 µg/ml) of triazine derivatives were introduced. Dimethyl sulfoxide was used as a control. After introduction of standard drug and

extracts, the plates were placed in a refrigerator at 8-10°C for proper diffusion into media. After two hrs of cold incubation, the Petri plates are transferred to incubator and maintained at 37° ± 2°c for 48hrs. after the incubation period, the Petri plates were observed for zone of inhibition by using venier scale. The results evaluated by comparing the zone of inhibition shown by the derivatives with standard drug. The results are the mean values of zone of inhibition measured in mm of data were shown in the table no's 6.4, 6.5 and 6.6.

RESULTS AND DISCUSSION:

A total of 6 compounds were synthesized and recrystallized by appropriate solvents. They were identified and characterized by various spectral methods. All the compounds characterization data were shown in the Table no. 6.1

Table 6.1 Characterization data of the synthesized derivative compounds (Scheme 1)

Compound code	Mol. Formula	Mol. Weight (g/mol)	M.P (°C)	% yield	Rf value*
3a	$C_{15}H_{15}N0_2$	273.35	203-205°C	77%	0.44
3b	$C_{15}H_{15}N0_2$	273.35	201-203°C	76%	0.44
3c	$C_{16}H_{17}NO_3S$	303.38	164-166°C	87%	0.54
3d	$C_{16}H_{17}NO_3S$	303.38	162-164°C	86%	0.45
3e	$C_{17}H_{19}N0_4S$	333.40	208-210°C	84%	0.54
3f	$C_{15}H_{15}N0_3S$	289.35	248-250°C	81%	0.64

*Mobile phase = Ethanol + Toluene (7:3)

ANTIBACTERIAL STUDIES OF THE SYNTHESIZED COMPOUNDS

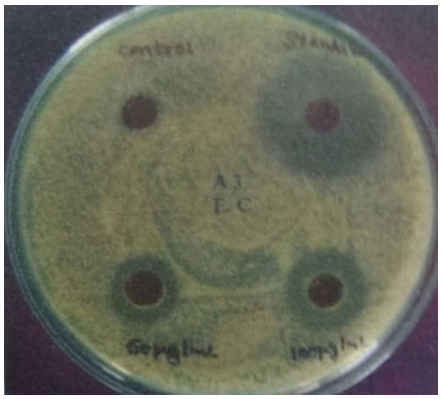
The synthesized 6 derivative compounds of scheme 1 were evaluated for antibacterial activity of cup plate method at the concentration of 50 µg/ml and 100 µg/ml using *Streptococcus Aureus, Bacillus pimilis* and *Escherichia coli*. Standard used was *streptomycin* and control are DMSO.

Table 6.2 zone of inhibition (in mm) obtained on bacteria

S.NO	Compound code	*Gram +ve*				*Gram –ve*	
		S.aureus		***B.pimilis***		***E.coli***	
		50 µg/ml	**100 µg/ml**	**50 µg/ml**	**100 µg/ml**	**50 µg/ml**	**100 µg/ml**
1.	**3a**	1	2	1	1.5	1	4
2.	**3b**	0	0	0	0	0	0
3.	**3c**	0	0	0	0	4	8
4.	**3d**	0	0	0	0	0	0
5.	**3e**	0.8	1.2	0.5	1.2	0	0
6.	**3f**	0	0	0	0	0	0
control	**DMSO**	0		0		0	
Standard	***Streptomycin 100µg/ml***	6		4		8	

(*) significant zone of inhibition bore size-10mm

Corrected zone of inhibition= obtained zone value – bore size



Zone of inhibition of *Escherichia coli*

Figure 6.3: Antibacterial activity of 3c

A total of 6 compounds were synthesized from scheme 1 were screened for anti-bacterial activity at concentrations 50 and 100 µg/ml. among the all compounds , 3C were found to have moderate activity against gram –ve E. coli.

Remaining all compounds was found to have less or no activity against gram +ve and gram –ve bacteria, compared to the standard streptomycin.

Anti-bacterial studies of the scheme synthesized derivative compounds

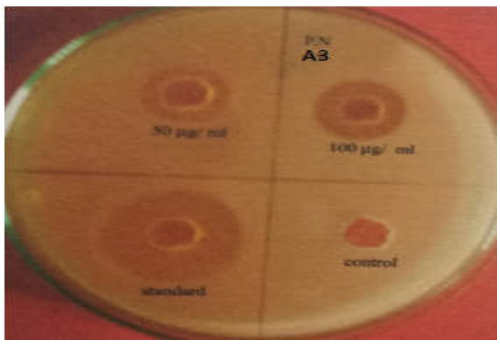
The synthesized derivative compounds of scheme 1 were evaluated for anti-fungal activity of cup plate method at the concentration of 50 µg/ml and 100 µg/ml using *Aspergillus Nigur* and *Penicillium Notatum.* Standard used was *miconazole nitrate* and control were DMF.

Anti-fungal studies of the synthesized derivative compounds (Scheme 1)

S.NO	Compound code	*Aspergillus Niger*		*Penicillium notatum*	
		50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
1.	**3a**	0	0	1	4
2.	**3b**	0	0	0	0
3.	**3c**	1	2	1	5*
4.	**3d**	0	0	0	4
5.	**3e**	0	0	0	0
6.	**3f**	0	0	0	0
control	**DMSO**	0	0	0	0
standard	**Miconazole nitrate**	14*		10*	

Table 6.5 zone of inhibition (in mm) obtained on fungi

(*) significant zone of inhibition bore size – 10 mm



Zone of inhibition of penicillium notatum

Figure 6.6: Anti-fungal activity of 3c

Summary:

A total of 6 compounds, synthesized from Scheme 1 were screened for anti-bacterial activity at concentrations 50 and 100 µg/ml. among all the compounds, 3c were found to have moderate activity against gram –ve E. coli. Remaining all compounds was found to be having less or no activity against gram +ve and gram –ve bacteria, compared to the standard streptomycin.

A total 6 compounds, from Scheme 1 were screened for anti anti fungal concentrations 50 and 100µg/ml, out of which only 3c has shown a moderate activity against penicillium notatum compared with standard miconazole nitrate. Remaining all compounds was found to be inactive against fungi.

Conclusion:

The compounds substituted chalcones were synthesized from aromatic aldehyde derivatives and characterized by spectral studies. The compounds were evaluated by antimicrobial activity. The results obtained indicate that a majority of compounds show only moderate insignificant activity. All the compounds showed dose dependant activity. The future prospect was too established newer anti-cancer and anti TB agents of chalcones synthesized derivatives.

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