SYNTHESIS OF HALOGENATED CHALCONES AND THEIR ANTIFUNGAL EVALUATION

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

INTEGRATED MASTER OF SCIENCE (HONS.)
in
CHEMISTRY
(Minor Subject: Biochemistry)

By
Navjot Singh Sarao
(L-2012-BS-84-IM)

Department of Chemistry
College of Basic Sciences and Humanities

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CERTIFICATE - II

This is to certify that the thesis entitled, **"Synthesis of halogenated chalcones and their antifungal evaluation"** submitted by **Navjot Singh Sarao** (L-2012-BS-84-IM) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Integrated Master of Science (Hons.)** in the subject of **Chemistry** (Minor subject: **Biochemistry**) has been approved by the Student's Advisory Committee along with the Head of the Department after an oral examination on the same.

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CERTIFICATE - I

This is to certify that the thesis entitled **"Synthesis of halogenated chalcones and their antifungal evaluation"** submitted for the degree of **Integrated Master of Science (Hons.)** in the subject of **Chemistry** (Minor subject: **Biochemistry**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Navjot Singh Sarao** (L-2012-BS-84-IM) under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

_____

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"Anytime you deny the acknowledgement of God you are undermining the entire basis of your own existence"

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Needless to say, errors and omissions if any are all mine.

_____

(Navjot Singh Sarao)

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ABSTRACT

Chalcone is a generic term used to describe compounds with the 1,3-diphenylprop-2-en-1-one framework. On synthetic ground, chalcones are synthesized by Claisen-Schmidt condensation reaction which involved the base mediated reaction of andifferent aldehydeswith different ketone in presence of strong bases like sodium hydroxide and potassium hydroxide. In the present study, series of novel halogenated chalcones (**1-14**) were synthesized through Claisen-Schmidt condensation reaction between different halogenated aldehydes and α, β-unsaturated halogenated ketonesunder basic conditions. The synthesized compounds were formulated as nano aqua emulsions using oleic acid as oil phase with Tween 20 as surfactant,under ultrasonic irradiation. Antifungal activity of prepared chalcone nanoemulsions was tested against various phytopathogenic fungi *viz. Pyriculriagrisea*, *Dreschslera oryzae*, *Colletotrichum falcatum and Ustilago hordei.* The results indicated that the various synthesized halogenated chalcones showed mild activity against various fungi.

Keywords: Aldehydes, acetophenones, halogenated chalcones, *Pyriculriagrisea*, *Dreschslera oryzae*, *Colletotrichum falcatum and Ustilagohordei*

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ਸਾਰ-ਅੰਸ਼

ਚਾਲਕੋਨ ਇੱਕ ਆਮ ਸ਼ਬਦ ਹੈ ਜਿਸ ਨੂੰ 1,3-ਡਾਈਫਿਨਾਈਲਪ੍ਰੋਪ-2-ਈਨ-1ਓਨ ਫਰੇਮਵਰਕ ਦਰਸਾਉਣ ਲਈ ਵਰਤਿਆ ਜਾਂਦਾ ਹੈ। ਸਿੰਥੈਟਿਕ ਤੌਰ ਤੇ, ਚਾਲਕੋਨਜ਼ ਨੂੰ ਕਲੇਜ਼ਰ-ਸਮਿੱਥ ਕਨਡਨਸੇਸ਼ਨ ਪ੍ਰਤੀਕਿਰਿਆ ਰਾਹੀਂ ਤਿਆਰ ਕੀਤਾ ਜਾਂਦਾ ਹੈ ਜਿਸ ਵਿੱਚ ਵੱਖਰੇ ਐਲਡੀਹਾਈਡਸ ਦੀ ਕਿਰਿਆ ਵੱਕਰੇ ਕਿਟੋਨਜ਼ ਨਾਲ ਸੋਡੀਅਮ ਹਾਈਡ੍ਰੋਕਸਾਈਡ ਜਾਂ ਪੋਟਾਸ਼ੀਅਮ ਹਾਈਡ੍ਰੋਸਾਈਡ ਦੀ ਮੌਜੂਦਗੀ ਵਿੱਚ ਕੀਤੀ ਜਾਂਦੀ ਹੈ। ਮੌਜੂਦਾ ਅਧਿਐਨ ਵਿੱਚ, ਨਵੇਂ ਹੈਲੋਜੀਨੇਟਡ ਚਾਲਕੋਨਜ਼ (1-14) ਨੂੰ ਵੱਖਰੇ ਹੈਲੋਜੀਨੇਟਡ ਐਲਡੀਹਾਈਡਸ ਅਤੇ ਵੱਖਰੇ ਹੈਲੋਜੀਨੇਟਡ ਐਸੀਟੋਟਿਨੋਨਜ਼ ਦੀ ਕਿਰਿਆ ਨਾਲ ਤਿਆਰ ਕੀਤਾ ਗਿਆ। ਫਿਰ ਉਤਪਾਦਕ ਕੰਪਾਊਂਡਸ ਨੂੰ ਨੈਨੋ ਇਮਲਸ਼ਨਜ ਦੇ ਤੌਰ ਤੇ ਤਿਆਰ ਕੀਤਾ ਗਿਆ ਜਿਵੇਂ ਓਲਿਕ ਐਸਿਡ ਨੂੰ ਤੇਲ ਦੇ ਪੜਾਅ ਦੇ ਨਾਲ ਟਵੀਨ 20 ਦੇ ਰੂਪ ਵਿੱਚ ਅਲਟਰਾ ਸੋਨਿਕ ਰਾਹੀਂ ਤਿਆਰ ਕੀਤਾ। ਤਿਆਰ ਕੀਤੇ ਚਾਲਕੋਨ ਨੈਨੋ ਇਮਲਸ਼ਜ ਦੀ ਉਲੀਨਾਸ਼ਕ ਗਤੀਵਿਧੀਆਂ ਨੂੰ ਵੱਖ-2 ਉਲੀਆਂ ਜਿਵੇਂ ਕਿ ਪਾਈਰੀਕੁਲੇਰਿਆ ਗ੍ਰਿਸੇ, ਡਰੇਸਕਲੈਗ ਔਰਜੀ, ਕਲੈਕਟੋਟ੍ਰਾਈਕਮ ਫਲਕੈਟਮ ਅਤੇ ਓਸਟੈਲਗੋ ਹਰਡਾਈ ਦੇ ਵਿਰੁੱਧ ਜਾਂਚ ਕੀਤਾ ਗਿਆ । ਸਾਰੇ ਉਤਪਾਦਨ ਕੰਪਾਊਂਡਸ ਨੇ ਉਲਿਆ ਵਿਰੁੱਧ ਹਲਕੀ ਗਤੀਵਿਧੀ ਵਿਖਾਈ ਸੀ ।

ਮੁੱਖ ਸ਼ਬਦ: ਐਲਡੀਹਾਈਡਸ, ਐਸੀਟੋਫਿਨੋਨਜ਼, ਹੈਲੋਜਿਨੇਟਰ ਚਾਲਕੋਨਜ਼, ਪਾਈਰੀਕੁਲੇਰਿਆ ਗ੍ਰਿਸੇ, ਡਰੇਸਕਲੈਗ ਔਰਜੀ, ਕਲੈਕਟੋਟ੍ਰਾਈਕਮ ਫਲਕੈਟਮ ਅਤੇ ਓਸਟੈਲਗੋ ਹਰਡਾਈ

ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ

ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ

CONTENTS

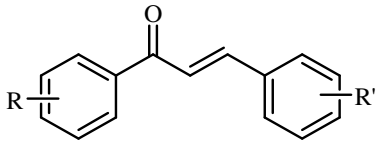
CHAPTER - I

INTRODUCTION

Phytopathogenic fungi and insect pests cause over 30% loss in crop yield worldwide. To increase yield and produce healthy crops, application of agrochemicals is often being used but occurrence of resistance to various commercially offered agrochemicals are posing threat to existing food security (FRAC 2013). Therefore, accelerated efforts are needed to overcome the problem, with addition of new molecules in the existing list of agrochemicals that may have the broad spectrum activity profile with different mode of action and most importantly the environmentally benign.

Chalcone (I) is the constituent of natural pigment of various plant species like *Angelica, Glycyrrhiza, Humulus, Scutellaria, Paratocarpus, Ficus, Dorstenia, Morus and Artocarpus*. These are widely used as traditional folk remedies indicating its bioactive relevance (Chavan *et al* 2016) and environment friendly existence. They are also abundant in edible plants and are considered to be synthon framework for flavonoids and isoflavnoids (Patil *et al* 2009).

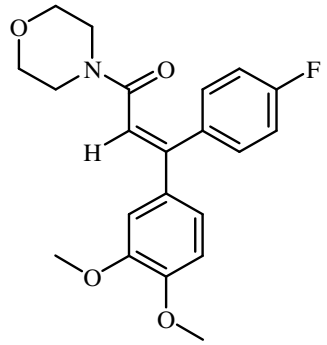
Chalcone is a generic term used to describe compounds with the 1,3-diphenylprop-2-en-1-one framework. The basic molecule contains two aromatic rings that are linked by an aliphatic three carbon chain. These are unsaturated ketones containing the reactive ketoethylenic group –CO-CH=CH- which is responsible for the variable bioactivity profile of the established moiety. On synthetic ground, chalcones are synthesized by Claisen-Schmidt condensation reaction (Sebti *et al* 2002, Clayden *et al* 2007), which involved the base mediated reaction of an aromatic aldehyde or ketone with an aliphatic ketone or aldehyde in presence of strong bases like sodium hydroxide and potassium hydroxide (Clayden *et al* 2007, Chavan *et al* 2016).



(I)

The reported profile of the synthetic derivatives indicated its vitality in various pharmacological and pathological fields for their various bioactivities *viz.* antioxidant (Aly *et al* 2014, Vazquez-Rodriguez *et al* 2013), anti-cancer (Singh *et al* 2012, Winter *et al* 2014), anti-inflammatory (Nowakowska 2007), antifungal (Hasan *et al* 2007), antibacterial (Chate *et al* 2012, Ritter *et al* 2015, Bozic *et al* 2014) and anti-malarial (Sinha *et al* 2013, Guantai *et al* 2011). On plant kingdom they are reported to work without exerting toxicological effect on plant tissues (Makovitzki *et al* 2007), but there is limited reported literature on its application to address the problem of modern agriculture. Flumorph (II) is the only example of

commercial fungicide containing chalcone moiety for use against *Phytophthora melonis* (Zhu *et al* 2007).



(II)

Association of halogen atoms on the chalcone template are known to augment their biopotential. Halogen present on chalcone exert a strong influence on the physiochemical profile of the molecule leading to the wide variation in its biological potential. Introduction of fluorine is a powerful strategy to optimize the properties of pharmaceutical and agricultural products (Leroux 2016). The functionalization of chalcones with halogens, for their evaluation against phytopathogenic fungi, is a demanding area of research for agriculturalists.

The hypothesis for the current research was made to derivatize halogenated chalcones with improved physiochemical properties for evaluation of antifungal activities against various phytopathogenic fungi. Thus, the work was planned with objectives:

1. To synthesize and characterize halogenated chalcones.
2. To evaluate fungicidal activity of synthesized halogenated chalcones.

The thesis runs into several chapters, namely, review of literature, materials and methods, results and discussion followed by summary.

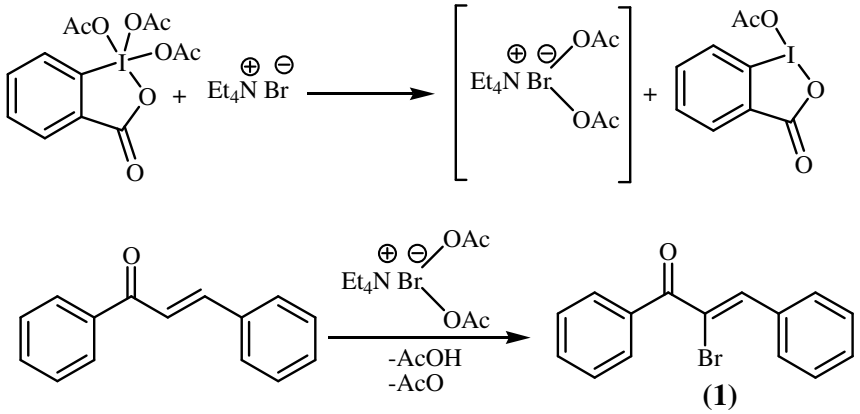
Since almost the entire investigation incorporated in this thesis is about synthesis of halogenated chalcones and their bioefficancy against *Drechsclera oryzae, Pyricularia grisea, Ustilago hordei* and *Collectotrichum falcatum.* The review of literature on synthesized halogenated chalcones and their biological activities was thought to be appropriate. This review is given in chapter II of the thesis. Various methods and techniques employed and different reagents and chemicals used during investigations are given in chapter III. In chapter IV, results of our investigations along with discussion have been described. Chapter V comprises the summary of the research work carried out.

CHAPTER – II

REVIEW OF LITERATURE

The classical bromination method of chalcones with Br_2 affording α,β-dibromo chalcones which can undergo dehydrobromintaion in presence of a base (e.g. Et_3N) to get the α -bromochalcones is widely used in literature (Rekhter *et al* 1995, Ducki *et al* 2009).

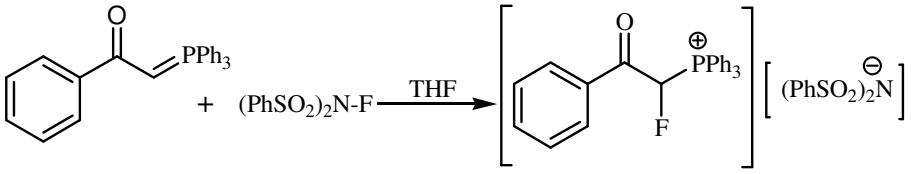
Ramanarayanan *et al* (2002) published the one-step procedure for the preparation of α -bromo- α, β -unsaturated carbonyl compounds from the corresponding α, β -unsaturated carbonyl compounds utilizing Et_4 NBr as a brominating agent in presence of Dess-Martin periodinane. When they applied their method on chalcone they got a mixture of a (Z/E)- α -bromo-chalcone but Z was the major isomer. Two acetate ligands of DMP firstly transfer to Et_4NBr forming tetraethylammonium [di(acyloxy)bromate] which then adds to the double bond of chalcone to form bromoacetoxylated intermediate. The acetate ion formed in the reaction acts as a base and abstracts the α -H furnishing the α -Br-chalcone (1).

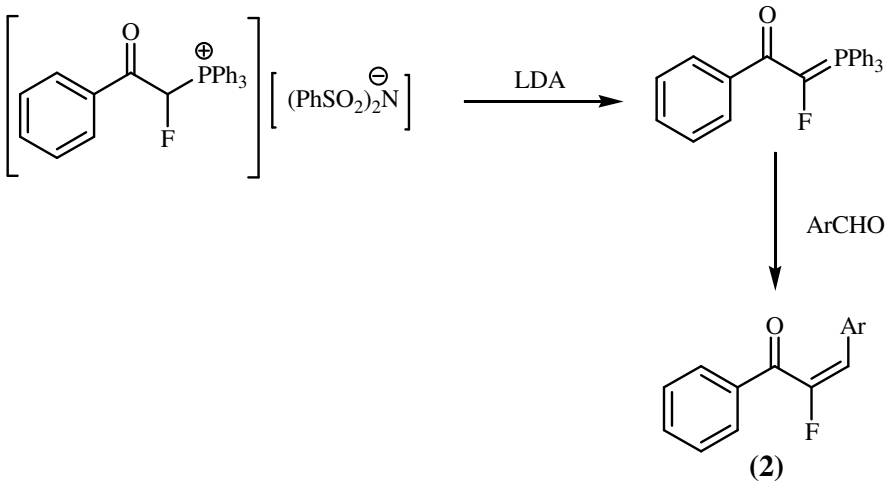


(1)

Scheme 1

Huang and Wang (2002) reported the synthesis of (E)-α-F-chalcones (2) by Wittig reaction *via* the corresponding α-F-substituted ylids. Ylid reacted with N-fluorodiphenylsulfonamide to form salt and then a strong base (lithium diisopropylamine, LDA) was added to form ylid smoothly. The α-F-ylid then treated with an aldehyde to give (E)-α-F-chalcones.



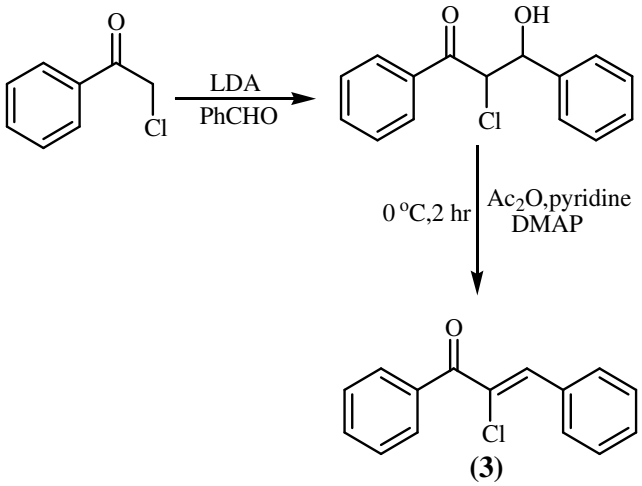


(2)

Ar = 4-$NO_2C_6H_4$, 4-ClC_6H_4, C_6H_5, 4-FC_6H_4, C_6H_5, 4-$CH_3C_6H_4$

Scheme 2

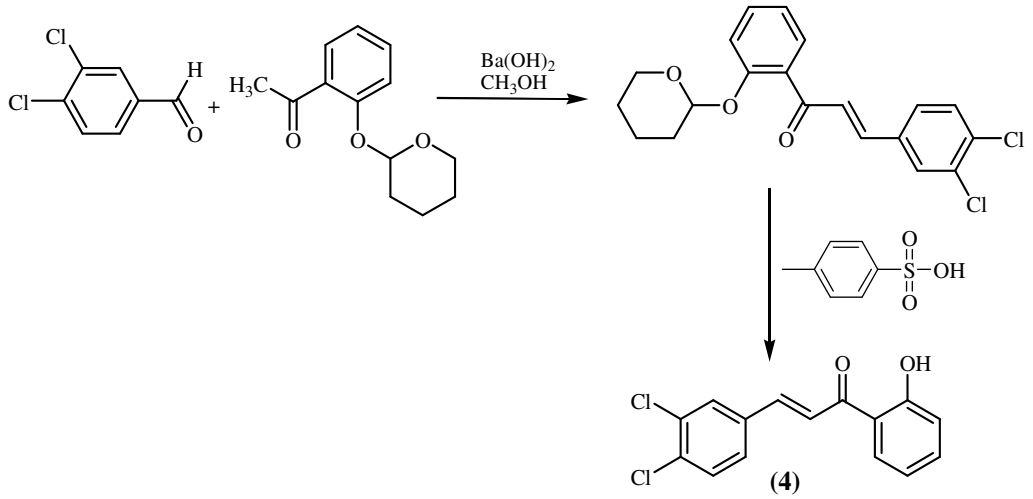
(*Z*)-α-chloro-α,β-unsaturated ketones (3) were synthesized by reacting α-chloro-β-hydroxyketones with acetic anhydride, pyridine and 4-dimethylaminopyridine (DMAP) as reported by Concellon and Huerta (2002). The α-Cl-acetophenone was treated with LDA or potassium hexamethyldisilazide (KHMDS) to get the enolate that was further reacted with benzaldehyde to get the α-chloro-β-hydroxyketone and elimination of water gave α-Cl-chalcone.



(3)

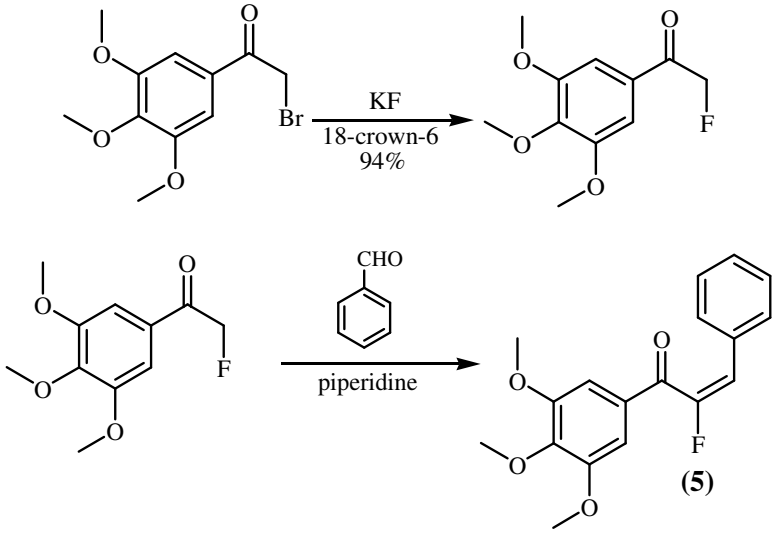
Scheme 3

Shen-Jeu *et al* (2005) reported synthetic chalcones as potential anti-inflammatory and cancer chemopreventive agents. Chalcones (4) were prepared by Claisen-Schmidt condensation of appropriate acetophenones with suitable aromatic aldehyde or by treating appropriate dihydrochalcone with suitable alkyl bromide or prepared in one-pot procedure involving acetophenone and convenient aromatic aldehyde using ultrasonic agitation on basic alumina.



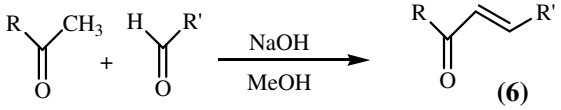
Scheme 4

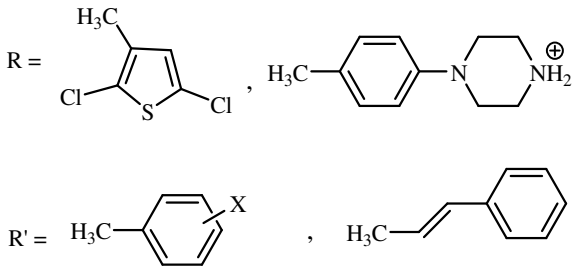
Series of (*Z*)-α-F-chalcones (5) were synthesized by Lawrence *et al* (2006) *via* aldol condensation between the α -F-acetophenone that was synthesized from the corresponding α -Br-acetophenone and an aldehyde in the presence of piperidine. The synthesized compounds showed potent cytotoxic and tubulin inhibitory properties.



Scheme 5

Tomar *et al* (2007) prepared various substituted chalcones (6) by reacting 4'-piperazino aceto- phenone or 3-acetyl-2,5-dichlorothiophene with aromatic aldehyde.



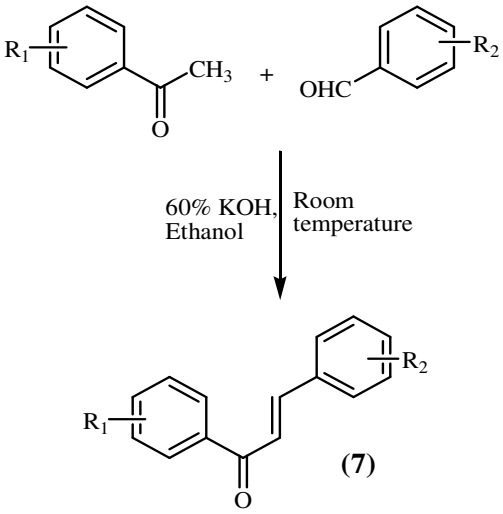


X = H, 3-CH_3, 4-OCH_3, 3,4,5-trimethoxy, 4-Cl, 3-NO_2

Scheme 6

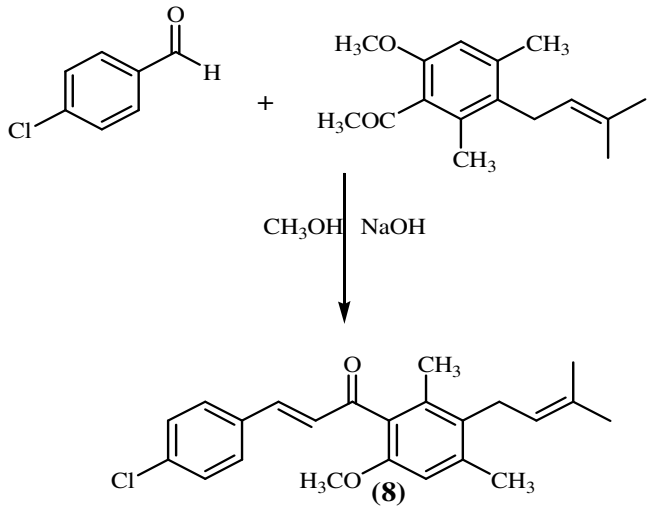
Halogenated chalcones were prepared by condensing halogenated hydroxyl acetophenones with halogen-substituted benzaldehyde in the presence of aqueous alcoholic alkali as reported by Hasan *et al* 2007. The antifungal activity of the synthesized compounds increased as the ring was substituted with more electronegative halogens such as fluorine.

Mandge *et al* (2007) reported the synthesis of chalcones (7) by condensation of appropriate aromatic ketones with substituted benzaldehydes using base as catalyst. The structure of all synthesized compounds were confirmed by IR, mass spectroscopy and elemental analysis.



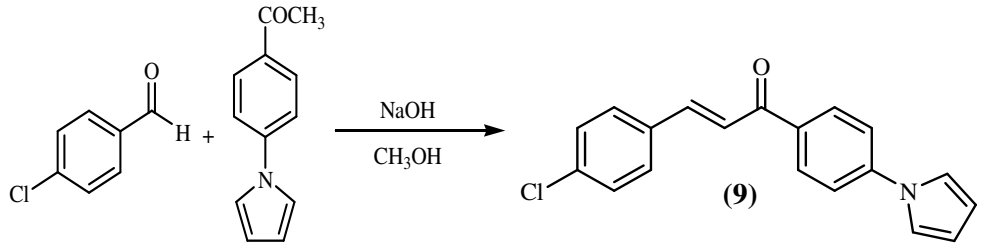
Scheme 7

Susanne *et al* (2008) reported synthesis of series of prenylated chalcones (8). The novel chalcones were evaluated for their cytotoxic and anti-oxidative activities.



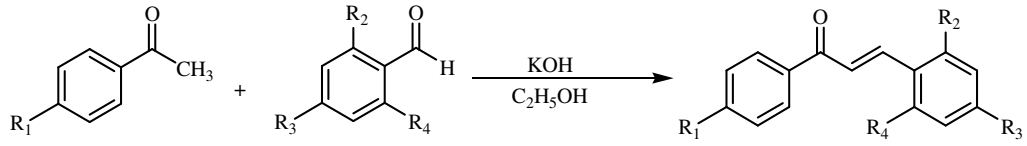
Scheme 8

Amit *et al* (2009) reported synthesis of series of chalcone derivatives (9). The new chalcone combination with artemisinin was evaluated *in vitro* for antimalarial activity against *Plasmodium falciparum.*



Scheme 9

Solution-phase parallel synthesis of substituted chalcones (10) and their antiparasitary activity against *Giardia lamblia* had been reported by Julio *et al* (2009). Substituted aromatic acetophenones and various benzaldehydes were condensed using the Claisen–Schmidt base-catalyzed reaction. Several chalcones showed *in vitro* antiparasitic activity against *Giardia lamblia.*

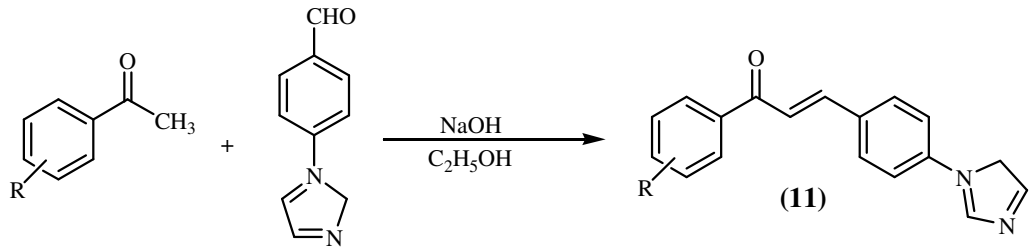


(10)

R_1= H, Cl, F, OH, OCH_3
R_2=R_3= H
R_4= H, CH_3, OCH_3

Scheme 10

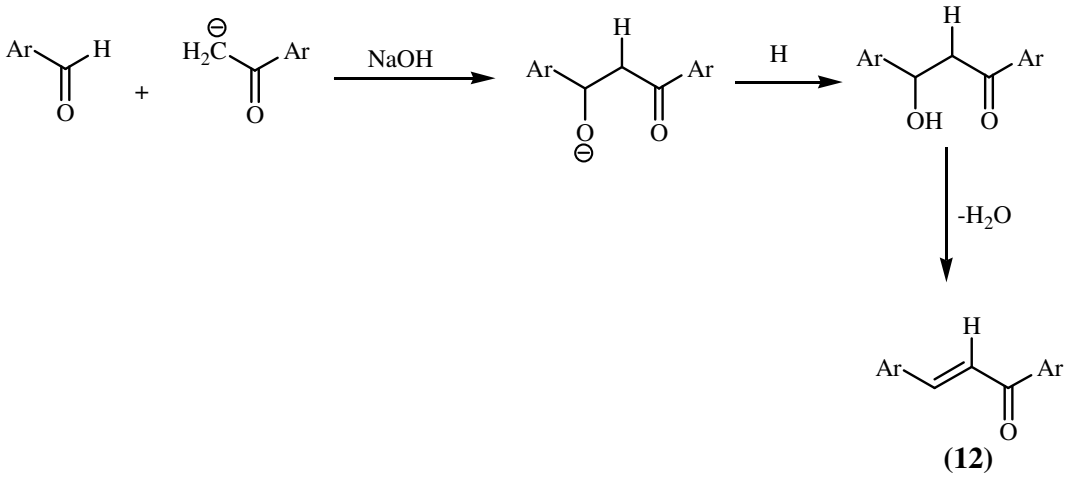
Tanvir *et al* (2009) have reported synthesis and biological activity of new 3-[4-(1*H*-imidazol-1-yl) phenyl]prop-2-en-1-ones (11). All the synthesized compounds were evaluated for their anti-leishmanial, anti-oxidant and antifungal activities. Some synthesized compounds showed good antifungal activity.



R = H, 2-Cl, 3-Cl, 2-F, 3-F, 4-OCH_3

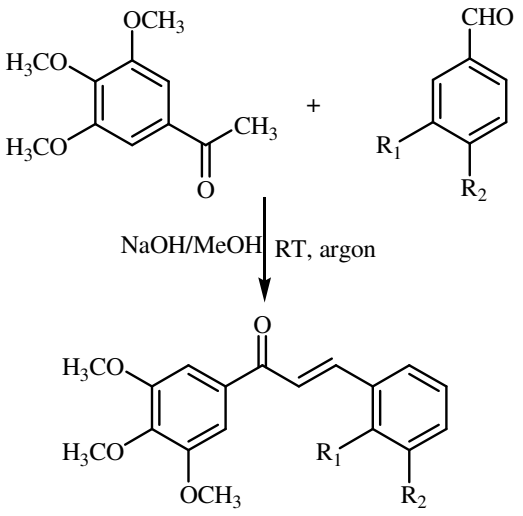
Scheme 11

Patil *et al* (2009) decribed the mechanism for synthesis of chalcones (12), its chemical modifications to flavonoids, flavanone, pyrazoles, oxazoles and pyrimidines. Patil and his co-workers described the antioxidant potential of chalcone, mechanism of antioxidant activity of chalcones and structure activity relationship of chalcone derivatives for antioxidant ability and different methods to evaluate antioxidant activity of chalcone, antiinflammatory, cytotoxic and antihyperglycemic activity of chalcones.



Scheme 12

Bonesi *et al* (2010) synthesized a series of chalcones (13) by aldolic condensation of 3,4,5-trimethoxy-acetophenone with appropriately substituted benzaldehydes.



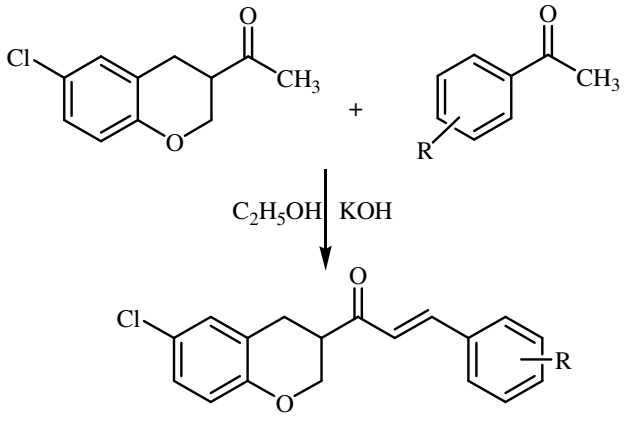
(13)

R_1 = H, OCH_3, OH, NO_2, F

R_2 = H, OCH_3, OH, NO_2

Scheme 13

Zohreh *et al* (2010) had reported the synthesis of novel chalcones (14) containing a 6-chloro-2H chromen-3-yl group. The target compounds were evaluated against the promastigote form of Leishmania major using MTT assay. All of the evaluated compounds have shown high *in vitro* antileishmanial activity at concentrations less than 3.0 µM. The results of cytotoxicity assessment against mouse peritoneal macrophage cells showed that these compounds display antileishmanial activity at non-cytotoxic concentrations.

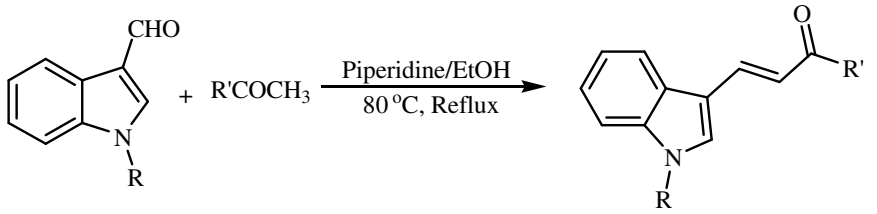


(14)

R= 2-F, 4-Cl, 1,2,3-trimethoxy

Scheme 14

Kumar *et al* (2010) synthesized indolyl chalcones (15) by reacting indol-3-carboxaldehyde and appropriate acetophenone in presence of piperidine under reflux.



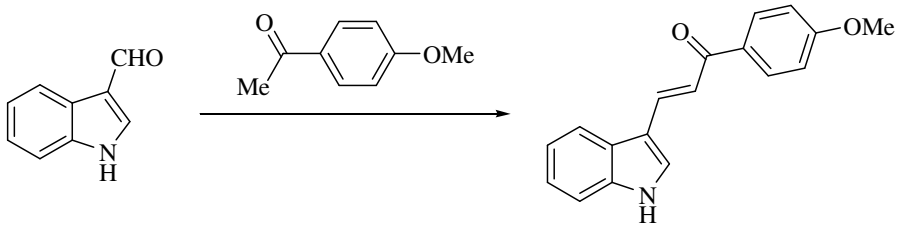
(15)

R= H, CH_3

R' = 4-OHC_6H_4, 4-$OCH_3C_6H_4$, 3,4-$(OCH_3)_2C_6H_3$, 3,4-$(OCH_2O)_2C_6H_3$, 4-FC_6H_4, 2-C_5H_4N

Scheme 15

Nassar (2010) gave method for aldol condensation reaction between 3-indolaldehyde and 4-methoxyacetophenone and afforded chalcone compound (16) and it was further derivatized. These compounds were screened in vitro for their bactericidal activity against Gram positive bacteria (*Staphylococcus aureus*) and Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeroginosa*) and for their fungicidal activity against *Aspergillus niger* and *Candida albicans*. All the screened compounds showed moderate to high bactericidal activity against *Staphylococcus aureus* and *Pseudomonas aeroginosa* as compared to ciprofloxacin.

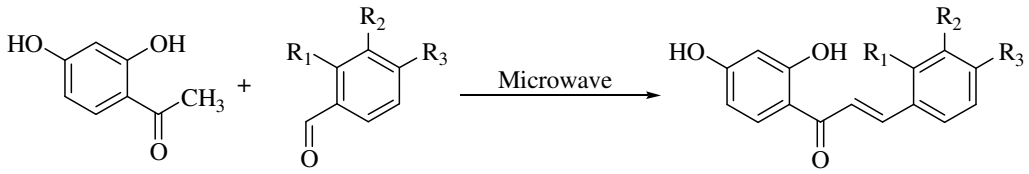


(16)

Scheme 16

Panchal *et al* (2011) reported the synthesis of chalcone 3-(Substitutedphenyl)-N-(4H-1, 2, 4-triazol-4-yl)acrylamide by condensation of substituted benzaldehyde with N-(4H-1,2,4-triazol-4-yl)acetamide under basic conditions. Some compound of the synthesized series exhibited promising anti-microbial and antifungal activity as compared to standard drugs.

A facile synthesis of some novel chalcones (17) by the condensation of variously substituted aromatic aldehydes and 2,4-dihydroxyacetophenone and their subsequent rapid one pot transformations to 2-aminobenzene-1,3-dicarbonitriles with malononitrile and morpholine had been described by Ameta *et al* (2011).



(17)

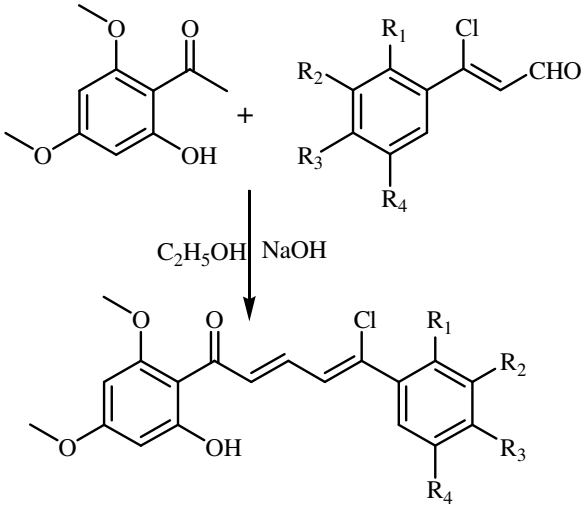
R_1= H,OH

R_2 = H, OCH_3, CH_3

R_3= H, OH CH_3, F

Scheme 17

Babasaheb *et al* (2011) have reported the synthesis and biological evaluation of β-chloro vinyl chalcones (18). All synthesized compounds were evaluated for their anti-inflammatory activity and antimicrobial activity. Most of compounds showed very good antibacterial and antifungal activity.

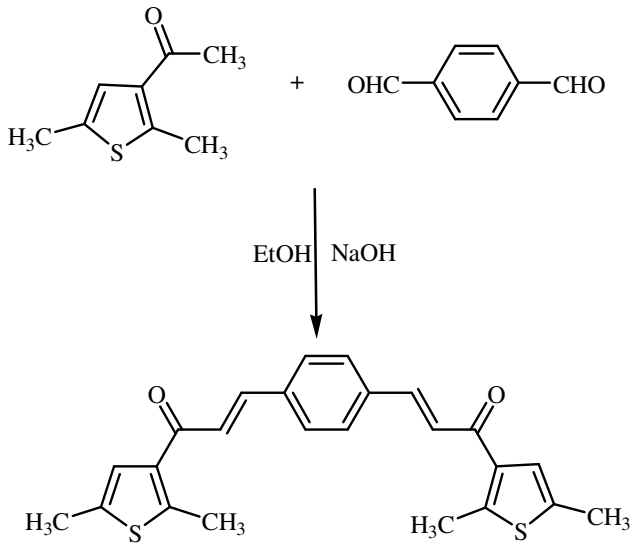


(18)

R_1, R_2. R_3, R_4 = Cl, Br, F

Scheme 18

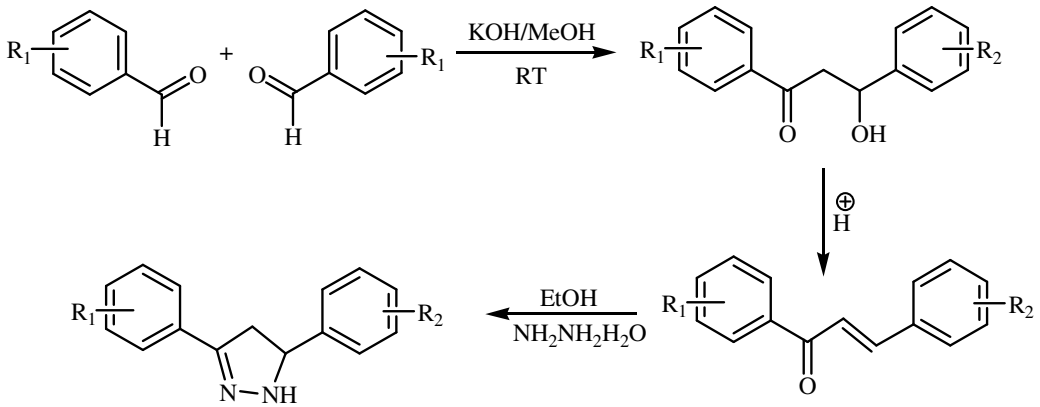
A chalcone (19) was prepared by the reaction of terephthalaldehyde with 3-acetyl-2,5-dimethylthiophene as reported by Asiri and Khan (2011). The anti-bacterial activity of these compounds were first tested *in vitro* by the disk diffusion assay against *Staphylococcus aureus, Streptococcus pyogenes* , *Salmonella typhimurium* and *Escherichia coli* and then the minimum inhibitory concentration (MIC) was determined with the reference of standard drug chloramphenicol. The results showed that the pyrazoline derivative was better at inhibiting growth of both types of bacteria compared to chloramphenicol.



(19)

Scheme 19

A new series of chalcones (20) and allylicchalcones (21) have been prepared by the Claisen-Schmidt condensation. A novel series of pyrazolic chalcones have been synthesized by the reaction of respective chalcones and hydrazine hydrate. All of the compounds have been tested for their anti microbial activities and antioxidant activities. The test compounds failed to show antibacterial properties or exhibited such properties poorly. Out of the compounds tested, some exhibited promising antioxidant activities as reported by Doan and Tran (2011).

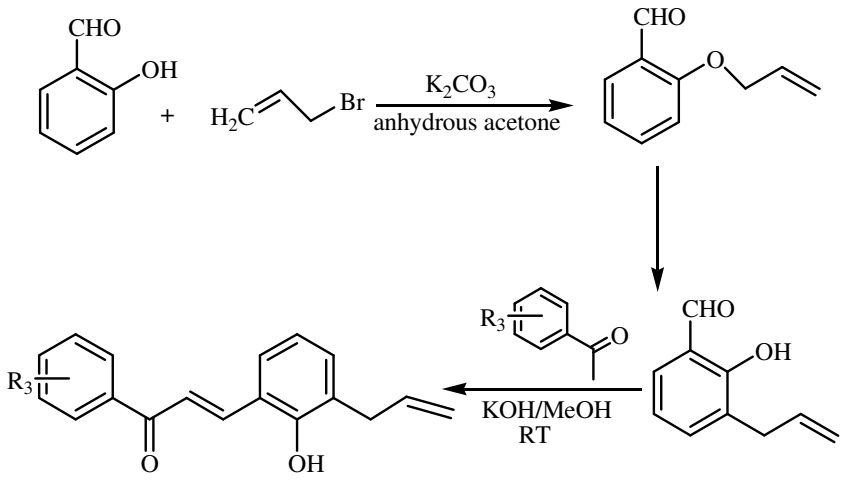


(20)

$R_{1=}$ 2-OH, 4-OCH_3, R_2= 3-OCH_3, 4-OCH_3

$R_{1=}$ 4-NO_2, R_2= 4-$N(CH_3)_2$

$R_{1=}$ 2-OH, R_2= 2-OCH_3, 4-OCH_3

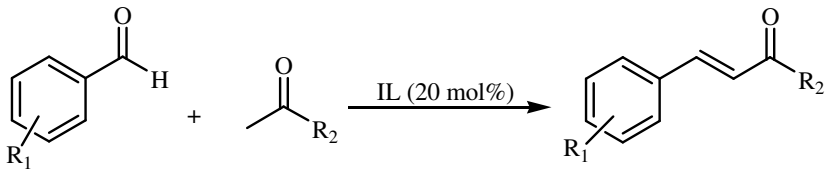


(**21**)

R_3= 2-OH, 5-CH_3, 4-NO_2

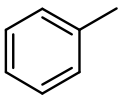
Scheme 20

Qian and Liu (2011) reported the synthesis of chalcones (22) *via* Claisen-Schmidht condensation between acetophenone ans benzaldehyde by using sulfonic acid-Functional ionic liquids (ILs). These proved to be a very active and leads to an 85-94 % yield of chalcones.



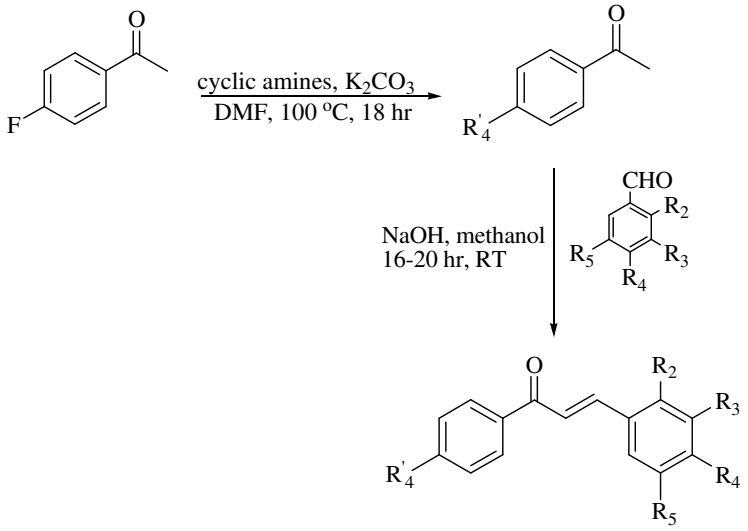
(**22**)

R_1= H, 4-NO_2, 4-Cl, 4-OCH_3, 3-OCH_3, 2-OCH_3

R_2 = CH_3, 

Scheme 21

Novel chalcone derivatives (23) were synthesized using Claisen-Schmidt condensation and their antimalarial activity against asexual blood stages of *Plasmodium falciparum* was determined by Yadav *et al* (2012). The presence of methoxy groups at position 2 and 4 in chalcone derivatives appeared to be favorable for antimalarial activity as compared to other methoxy-substituted chalcones.

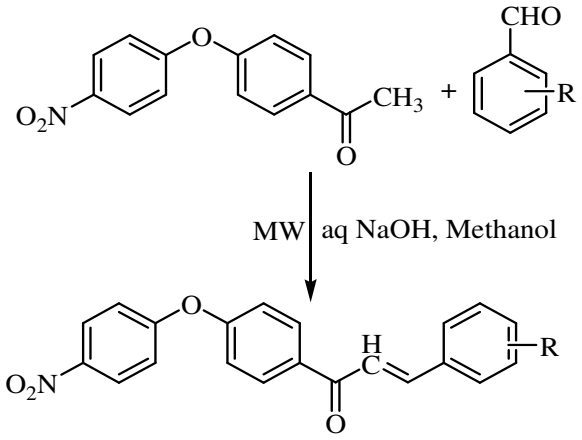


(23)

R_2 = H,	R_3 = H,	R_4= OCH_3,	R_5 = H
R_2 = OCH_3,	R_3 = H,	R_4= OCH_3,	R_5 = H
R_2 = OCH_3,	R_3 = H,	R_4= H,	R_5 = OCH_3
R_2 = H,	R_3 = OCH_3,	R_4= OCH_3,	R_5 = H
R_2 = H,	R_3 = OCH_3,	R_4= OCH_3,	R_5 = OCH_3

Scheme 22

Gothwal and Shrivastava (2012) developed a method for the synthesis of a series of chalcones (24) by the treatment of 1-(4-(4-nitrophenoxy)phenyl)ethanone with various substituted aldehydes in NaOH under microwave irradiation in 80-85% yield. These compounds were evaluated for their antimicrobial activities against bacteria *Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis, Candida albicans* and *Aspergilus fumigatu.* Some of the compounds showed potential activity against selected organisms.

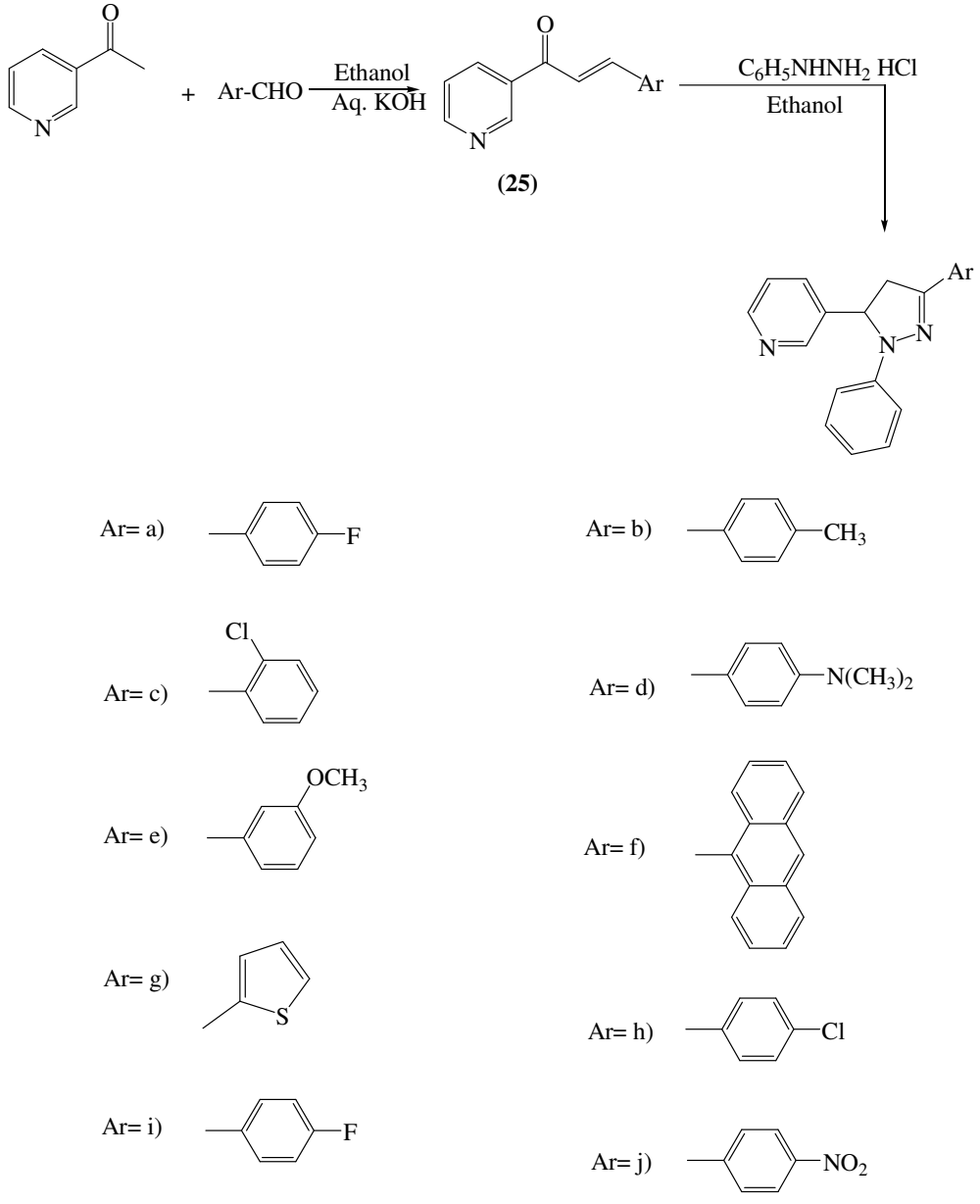


(24)

R = H, 4-$N(CH_3)_2$, 4-Cl, 3,4-(OCH_3), 4-OCH_3

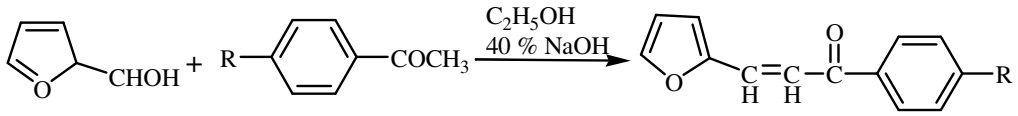
Scheme 23

Jyothi *et al* (2012) also synthesized some novel pyrazolines having antimicrobial activity. In first step, 3-acetylpyridine and respective aldehyde was reacted in aqueous KOH (40%) in ethanol to afford chalcones (25). Then in second step, the chalcones were reacted with phenyl hydrazine hydrochloride in ethanol under refluxing conditions to get the final product namely 1-phenyl-3-(3'-pyridyl)-5-(substitutedphenyl)-2-pyrazoline derivatives. These were then screened for their antibacterial and antifungal activity and it was observed that pyrazoline containing flouro substitution para position on phenyl ring enhanced both antibacterial and antifungal activities.



Scheme 24

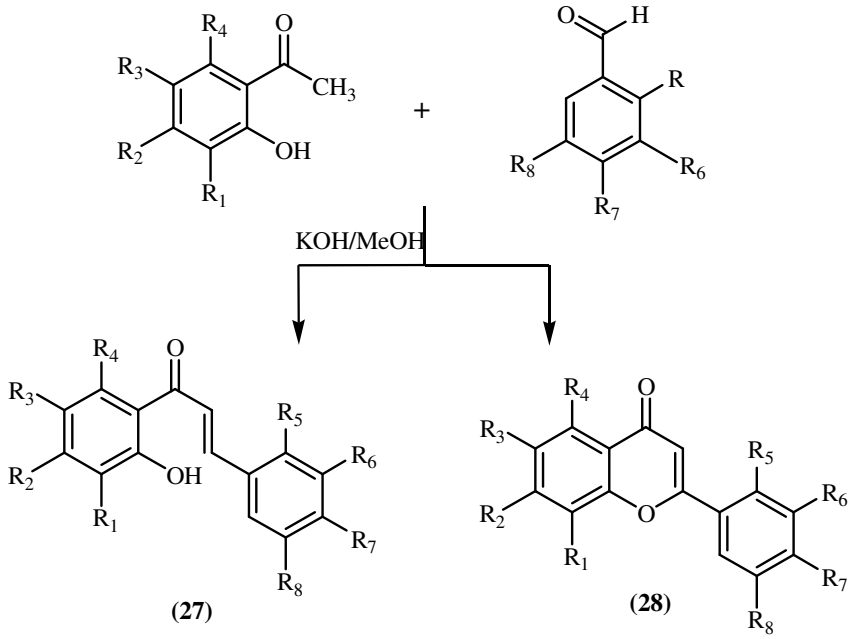
Joshi *et al* (2012) synthesized chalcones (26) by treatment of furan-2-carbaldehyde with different acetophenones by Claisen-Schmidt condensation. Then various pyrazoline derivatives were prepared by refluxing chalcones with phenyl hydrazine/hydrazine hydrate in ethanolic solution. The structures of newly synthesized compounds were established on the basis of their spectral data. All synthesized compounds were screened for their antimicrobial activity against some bacteria and fungi.



R= NH_2, Br, NO_2, Cl, F, CH_3

Scheme 25

Albogami *et al* (2012) had been reported the facile and highly efficient microwave-assisted synthesis of functionalized chalcones (27) and flavanones (28) based on the Claisen-Schmidt condensation reaction.



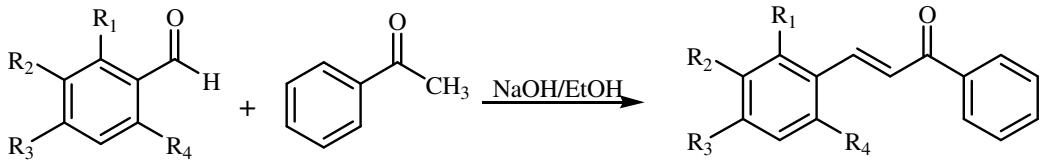
(27) **(28)**

R_1 = H, Cl
R_2 = H, OMe
R_3 = H, OMe, CH_3, Cl, Br
R_4= H, Cl
R_5 = H, OMe
R_6 = H,OMe
R_7 = H, OMe, Cl
R_8 = H, OMe

Scheme 26

Arora *et al* (2012) had been reported a series of chalcones of 2-acetyl naphthalene and substituted aryl aldehydes were synthesized and evaluated for antimicrobial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeuroginosa* and *Streptococcus pyogenes* and antifungal activity against *Aspergillus flavus, Aspergillus fumigatus, Candida albicans* and *Penicillium marneffei*. Results had shown that all the chalcones possess mild antifungal activity.

Chalcones (29) *via* Claisen-Schmidt condensation reaction with acetophenone and 2-nitrobenzaldehyde, 3-nitrobenzaldehyde, 4-nitrobenzaldehyde, 2,6-dichlorobenzaldehyde and 3,4-dichlorobenzaldehyde in presence of aqueous NaOH (0.05M) in ethanol, at room temperature had been reported by Alacorn *et al* (2013).



(29)

R_1= NO_2, Cl, H

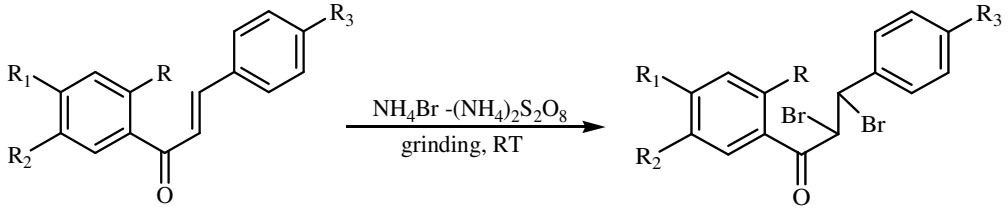
R_2= NO_2, Cl, H

R_3= NO_2, Cl, H

R_4= NO_2, Cl, H

Scheme 27

Selective bromination of chalcones and 2-hydroxychalcones has been carried out with ammonium bromide and ammonium persulphate using grinding technique at RT under aqueous moist conditions to give α,β-dibromochalcones and α,β-dibromo-2-hydroxychalcones respectively has been reported by Jakhar and Makrandi (2013).



R= H,OH

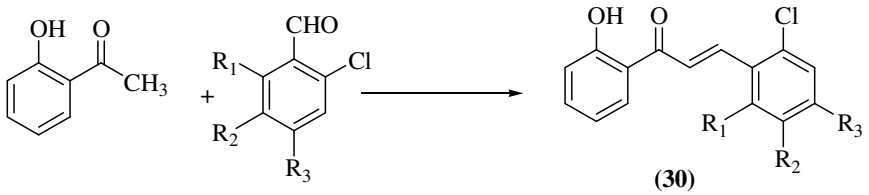
R_1= H, NO_2 Br, OCH_3

R_2= H, CH_3

R_3= H,OCH_3, Cl, CH_3

Scheme 28

A novel method for the synthesis of 2-hydroxy chalcones (30) *via* Clasien-Schmidt is introduced using silica gel supported piperidine as an alternative base had been reported by Dhananjayulu *et al* (2014).

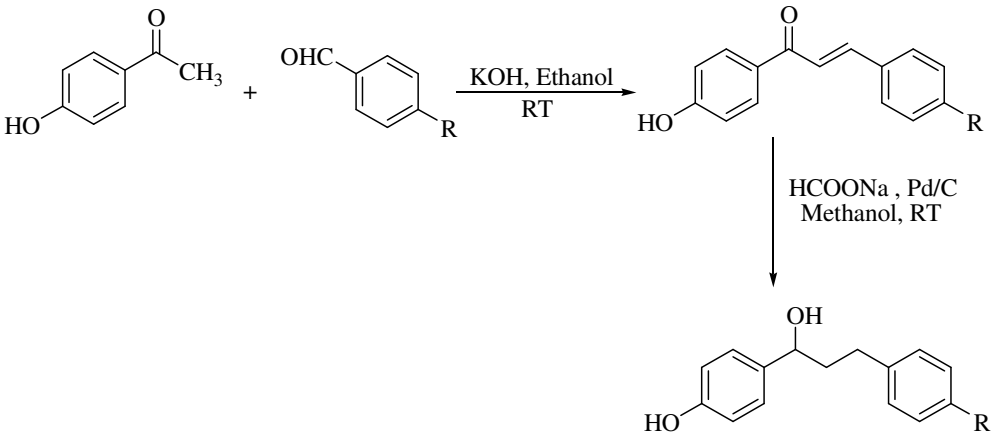


(30)

R_1 = H, R_2 = H, R_3 = Cl
R_1 = H, R_2 = NO_2, R_3 = H
R_1 = F, R_2 = H, R_3 = H

Scheme 29

Schmidt condensation reaction and subsequent one step reduction to tetrahydro chalcones (31) had been reported by Tailor (2014) by using sodium formate and 5% Pd/C in methanol at room temperature. Antifungal activity against *P. chrsogenum, A. nigar, A. fluvus* and *R. oligospora* was evaluated by using agar diffusion. α,β- unsaturated carbonyl functionality imparts very significant role in antifungal activity. Chalcones had more potential than corresponding tetrahydrochalcones.

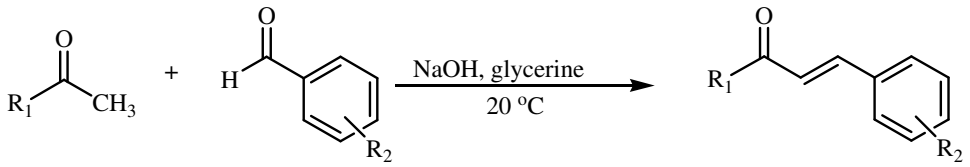


(31)

R = H, CH_3, OCH_3, F, CI, Br

Scheme 30

A green method was developed for the synthesis of chalcones (32) using glycerin as solvent as reported by Ritter *et al* (2015). Subsequently, the potential microbiology activity of these molecules was evaluated by testing them against the gram-positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis*, the gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and the fungus *Candila albicans* which includes three clinical strains of *Candila albicans* from human oral cavities. The result showed that some chalcones exhibited moderate inhibitory activity, the most prominent being those acting against the fluconazole-resistant strains of *Candila albicans*.



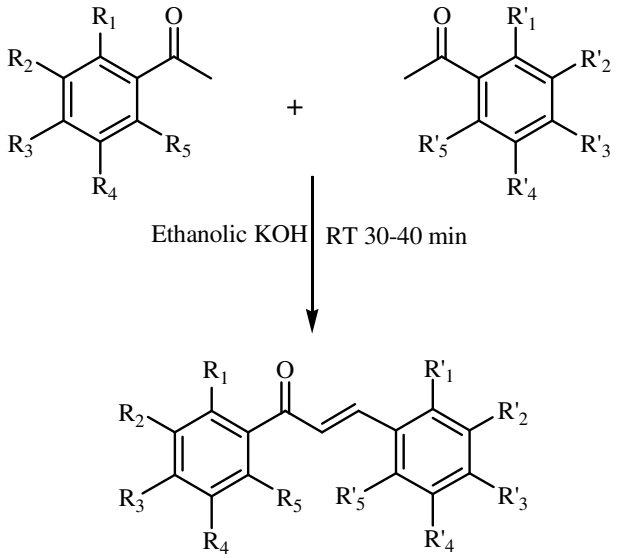
(32)

R_1 = Phenyl or 2-thiophene

R_2 = H, 4-OCH_3, 4-F, 4-Cl, 4-Br, 4-CH_3

Scheme 31

The new halogenated chalcones (33) designed and synthesized by Jain *et al* (2014) based on the bioisosteric replacement of a known ligand, were well docked onto the binding pocket of caspase enzyme and interacted with crucial amino acid residues.

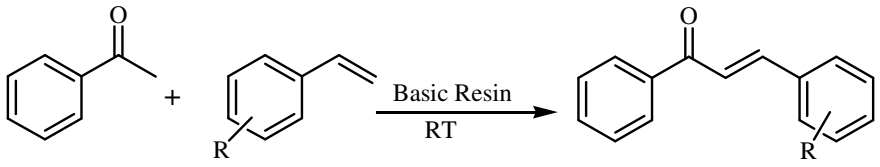


(33)

R_1 = H	R'_1 = H, Br, Cl, F, OCH_3
R_2 = H	R'_2 = H, OCH_3
R_3 = H, Br, Cl, OCH_3	R'_3 = H, OCH_3
R_4 = H	R'_4 = H, OCH_3
R_5 = H, OCH_3	R'_5 = H, Br, Cl, F, OCH_3

Scheme 32

Ezhilarasi *et al* (2015) had been reported the condensation of acetophenone with substituted benzaldehyde in the presence of anion exchange resin at ambient temperature to afforded the chalcones (34).

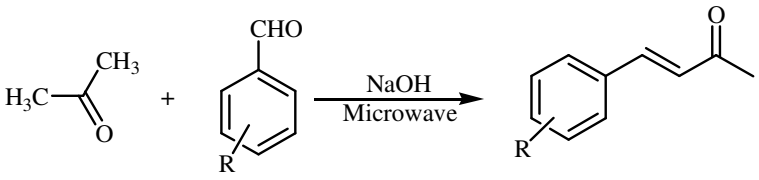


(34)

R = NO_2, CN, CH_3

Scheme 33

Microwave irradiation of different aldehydes with acetone produces benzalacetones (35) selectively without self-condensation product in very short reaction times and good yields as reported by Rayar *et al* (2015).



(35)

R = H, 4-CH_3, 4-tertbutyl, 4-F, 4-Br, 4-OCH_3, 3-Cl

4-NO_2, 3,4-dimethoxy, 5-Cl-3-NO_2

Scheme 34

CHAPTER III

MATERIALS AND METHODS

Chemical and reagents

The chemicals and reagents used were of AR (Analytical reagents) grades. List of chemicals and reagents used are given below:

i. Acetophenone - Sisco Research Laboratories Private Limited, Mumbai
ii. Acetone - Sisco Research Laboratories Private Limited, Mumbai
iii. 4-Bromoacetophenone - SpectroChem Private Limited
iv. 3-Bromobenzaldehyde - SpectroChem Private Limited
v. 4-Bromobenzaldehyde - SpectroChem Private Limited
vi. 4-Chloroacetophenone - Sisco Research Laboratories Private Limited, Mumbai
vii. 2-Chlorobenzaldehyde - Loba Chemie Private Limited, Mumbai
viii. 3-Chlorobenzaldehyde - Spectro Chem Private Limited
ix. 4-Chlorobenzaldehyde - Sisco Research Laboratories Private Limited
x. Chloroform - Sisco Research Laboratories Private Limited, Mumbai
xi. Dichloromethane - Sisco Research Laboratories Private Limited, Mumbai
xii. 4-Flouroacetophenone - SpectroChem Private Limited
xiii. 4-Flourobenzaldehyde - SpectroChem Private Limited
xiv. Furfuraldehyde - Loba Chemie Private Limited, Mumbai
xv. Methanol - Sisco Research Laboratories Private Limited, Mumbai
xvi. Sodium Hydroxide - Ranbaxy Laboratories Limited, SAS Nagar, Punjab
xvii. Oleic Acid - Loba Chemie Private Limited, Mumbai
xviii. Tween 20 - S.D. Fine Chemicals Limited, Mumbai

3.1 Synthesis of halogenated chalcones

The chalcones were synthesized by reacting the appropriate acetophenones with halogenated benzaldehydes.

3.1.1 3-(4-Chlorophenyl)-1-phenylprop-2-en-1-one (1)

To a solution of acetophenone (1.24 ml, 0.01 mole) in 10 ml methanol taken in 50 ml flask, 5 ml of 6 M aqueous solution of NaOH was added with constant stirring. *p*-Chlorobenzaldehyde (0.14 g, 0.01 mole) taken in separate beaker was dissolved in minimum quantity of methanol. This solution was added drop wise to the above mixture, with stirring for further 15-20 minutes. TLC was used to monitor the progress of the reaction. The solvent was evaporated and light yellow crude solid obtained was recrystallized from chloroform to get pure product of 3-(4-chlorophenyl)-1-phenylprop-2-en-1-one (1).

3.1.2 1,3- Bis(4-chlorophenyl)prop-2-en-1-one (2)

NaOH (6 M, 1.68 g) was added to a solution of 4-chloroacetophenone (1.40 ml, 0.01

mole) dissolved in 10 ml methanol taken in 50 ml of flask, while stirring. Then solution of *p*-chlorobenzaldehyde (0.14g, 0.01mole), prepared in separate beaker by dissolving it in minimum quantity of methanol, was added to above solution drop wise. It was allowed to stir for another 20 minutes and pale yellow colored precipitates were formed which were filtered and recrystallized from chloroform. Thin layer chromatography was used to check the purity of the compound.

3.1.3 3-(4-Fluorophenyl)-1-phenylprop-2-en-1-one (3)

6 M NaOH (5ml) was added to a solution of acetophenone (1.24 ml, 0.01 mole) in 10 ml methanol. The mixture was allowed to react with 5 ml solution of 0.01 mol (1.10 ml) of 4-flourobenzaldehyde following the same procedure as in 3.1.1. The whole reaction was carried out at 25°C. The crude product was recrystallized from chloroform to get cream colored 3-(4-fluorophenyl)-1-phenylprop-2-en-1-one (3).

3.1.4 1,3-Bis(4-fluorophenyl)prop-2-en-1-one (4)

1,3-Bis(4-fluorophenyl)prop-2-en-1-one (4) was prepared by stirring 4-fluoroacetophenone (1.20 ml, 0.01 mole) and 4-flurobenzaldehyde (1.10 ml, 0.01 mole) in methanol using aqueous NaOH solution as catalyst, at room temperature, according to the general procedure as of 3.1.1.

3.1.5 3-(3-Chlorophenyl)-1-(4-fluorophenyl) prop-2-en-1-one (5)

3-Chlorobenzaldehyde (1.20 ml, 0.01mole) was made to react with 4-fluoroacetophenone (1.20 ml, 0.01 mole) in methanol using aqueous NaOH as catalyst, at room temperature following the same procedure as of 3.1.1, to get 3-(3-chlorophenyl)-1-(4-fluorophenyl) prop-2-en-1-one (5).

3.1.6 3-(2-Chlorophenyl)-1-(4-fluorophenyl) prop-2-en-1-one (6)

2-Chlorobenzaldehyde (1.75 ml, 0.01mole) and 4-flouroacetophenone (1.20 ml, 0.01mole) reacted in the presence of NaOH (6 M, 1.68 g) in methanol in 200 ml beaker as in the experiment 3.1.1. Yellow colored crude solid obtained was recrystallized from chloroform to get pure 3-(2-chlorophenyl)-1-(4-fluorophenyl) prop-2-en-1-one (6).

3.1.7 1-(4-Bromophenyl)-3-(4-fluorophenyl) prop-2-en-1-one (7)

4-Fluroacetophenone (1.20 ml, 0.01 mole) and 4-bromobenzaldehyde (1.85 g, 0.01mole) were made to react in a methanolic solution using aqueous NaOH as catalyst, at room temperature by simple stirring (as in Experiment no. 3.1.1). The crude solid obtained was recrystallized from chloroform to get 1-(4-bromophenyl)-3-(4-fluorophenyl) prop-2-en-1-one (7).

3.1.8 3-(3-Bromophenyl)-1-(4-bromophenyl) prop-2-en-1-one (8)

3-(3-Bromophenyl)-1-(4-bromophenyl)prop-2-en-1-one (8) was obtained from 4-bromoacetophenone (0.01 mole, 2 g) and 3-bromobenzaldehyde (0.01 mole, 1.17 ml) using aqueous NaOH as catalyst (methodology same as in 3.1.1).

3.1.9 1-(4-Bromophenyl)-3-(2-chlorophenyl) prop-2-en-1-one (9)

2-Chlorobenzaldehyde (0.01 mole, 1.75 ml) and 4-bromoacetophene (0.01 mol, 2 g) reacted in the presence of NaOH (6 M) in methanol in 200 ml beaker as in the experiment 3.1.1. Yellow colored solid obtained was recrystallized from chloroform to get pure 1-(4-bromophenyl)-3-(2-chlorophenyl) prop-2-en-1-one (9). Thin layer chromatography was used to check the purity.

3.1.10 1-(4-Bromophenyl)-3-(3-chlorophenyl) prop-2-en-1-one (10)

4-Bromoacetophenone (2 g, 0.01mole) was dissolved in 10 ml of methanol in a flask and mixed with 5 ml of aqueous solution of 6 M NaOH (1.68 g). 3-Chlorobenzaldehyde (1.20 ml, 0.01 mole) was dissolved in minimum quantity methanol in a separate beaker. This solution was added drop wise to the above reaction mixture which was stirred at room temperature for 20 minutes. The pale yellow colored solid obtained was recrystallized from chloroform to get pure 1-(4-bromophenyl)-3-(3-chlorophenyl) prop-2-en-1-one (10) and was then, dried in folds of filter paper. The thin layer chromatography was used to check the purity of the prepared compound.

3.1.11 3-(4-Bromophenyl)-1-(4-fluorophenyl)prop-2-en-1-one (11)

4-Fluorobenzaldehyde (1.10 ml, 0.01 mole) was made to react with 4-bromoacetophenone (2 g, 0.01 mole) in methanol using aqueous NaOH (1.68 g) as catalyst, at room temperature and same procedure was followed as of 3.1.1, to get 3-(4-Bromophenyl)-1-(4-fluorophenyl)prop-2-en-1-one (11). The light yellow colored solid product obtained was recrystallized from chloroform. The thin layer chromatography was used to check the purity of the prepared compound.

3.1.12 1,3-Bis(4-bromophenyl) prop-2-en-1-one (12)

After dissolving 4-bromoacetophenone (2 g, 0.01 mole) in 10 ml methanol in a 200 ml flask, an aqueous solution of 6 M NaOH (1.68 g) was added and the resulting mixture was stirred at room temperature. Then, 4-bromobenzaldehyde (1.85 g, 0.01 mole) dissolved in minimum quantity of methanol taken in a beaker was added in the reaction mixture followed by 30 minutes stirring. The resulted yellowish solid product was filtered which was dried in folds of filter paper to get the pure product 1, 3-bis(4-bromophenyl)prop-2-en-1-one (12). Thin layer chromatography was used to confirm the purity of the compound.

3.1.13 1-(4-Fluorophenyl)-3-(furan-2-yl) prop-2-en-1-one (13)

4-Fluroacetophenone (0.01 mole, 1.20 ml) and Furfuraldehyde (0.01 mole, 0.82 ml) were made to react in a methanolic solution using aqueous NaOH as catalyst, at room temperature by simple stirring (as in Experiment no. 3.1.1). The crude solid obtained was recrystallized from chloroform to get 1-(4-fluorophenyl)-3-(furan-2-yl) prop-2-en-1-one (13).

3.1.14 1-(4-Bromophenyl)-3-(furan-2-yl) prop-2-en-1-one (14)

4-Bromoacetophenone (2 g, 0.01 mole) dissolved in 10 ml of methanol in 200 ml

flask, to which 5 ml of aqueous solution of NaOH (1.68 g, 6 M) was added. Then, this mixture was placed on the stirrer, to which the solution of furfuraldehyde (0.82 ml, 0.01 mole) dissolved in minimum quantity of methanol in a beaker and added drop wise to the above prepared solution. The mixture was allowed to continue stirring for 20 minutes. The dark brown colored solid product was obtained, which was recrystallized from chloroform to obtain pure product (*E*)-1-(4-bromophenyl)-3-(furan-2-yl) prop-2-en-1-one. The purity of the compound was confirmed by thin layer chromatography (TLC).

3.2 Preparation of nano emulsions of synthesized halogenated chalcones

Nano emulsions were formed by sonication method. 100 mg of the compound (1-14) was taken in the test tube and 1 ml of oleic acid was added. Then, it was heated gently on the hot plate to dissolve the compound in oleic acid. 5-6 ml of the acetone was added to the test tube to increase its volume and to make it less concentrated. 3 ml of Tween 20 dissolved in 47 ml of distilled water was taken in a 100 ml flask which was placed in sonicator. To the flask, solution of test tube was added dropwise. Sonication was continued for another 30 minutes to get the nano emulsions of respective chalcones (1-14).

3.3 Screening of antifungal activity

The synthesized halogenated chalcones were screened *in vitro* for their antifungal potential against four test fungi *viz. Pyriculria grisea*, *Dreschslera oryzae*, *Colletotrichum falcatum* and *Ustilago hordei* by spore germination inhibition technique. The results have been expressed in terms of ED_{50} values *i.e.* the effective dose at which 50 percent inhibition has occurred.

3.3.1 Collection, isolation and maintenance of cultures

Source:

The spores/cultures of the four test fungi were obtained from following sources:

P. grisea - Blast of rice

D. oryzae - Brown leaf spot of Rice

C. falcatum - Red rot of sugarcane

U. hordei - Loose smut of barley

3.3.2 Isolation of fungus

Diseased plant samples except loose smut of barley were collected from experimental areas of Punjab Agricultural University, Ludhiana and washed in running tap water for 5-10 min. The infected parts were cut into small pieces with the help of sterilized blade and sterilized with mercuric chloride solution (0.1 %) for one minute followed by three washings with sterilized distilled water. The sterilized infected pieces were transferred to Potato dextrose agar (PDA) medium (200 g peeled potato, 20 g dextrose and 20 g agar in 1 litre of water) slants under aspetic conditions. The test tubes were incubated at 25±1ºC in order to get good growth of fungus. The cultures so obtained were purified and maintained by further sub

culturing on PDA slants and keeping them in refrigerator.

3.3.3 Preparation of stock solution

The stock solutions (2000 µg ml^{-1}) were taken as such as prepared in section 3.2 of each compound (1-14) on active ingredient basis and were kept in refrigerator till further use. The required dilutions of 1000, 500, 250, 100, 50, 25 and 10 µg ml^{-1} were subsequently made in test tubes from the stock solution by adding distilled water as and when required.

For the comparison of the results of the test compounds, the commercial standards were taken as positive control. Propiconazole (Tilt 25 EC) was used against *D. oryzae*, *C. falcatum* and *P. grisea*. The standard solution of the fungicide was made on active ingredient basis, 1 ml of propiconazole was diluted to 1000 ml to obtain 250 µg ml^{-1} of the fungicide concentration. The solution was serially diluted by applying normality equation to the concentration of 100, 50, 25, 10 and 5 µg ml^{-1} respectively and were tested against the test tube. Against the *U. hordei*, Tebuconazole (Raxil 60 FS) was used as standard. On the basis of active ingredient 400 µg ml^{-1} of the stock solution was prepared by dissolving 10 mg of fungicide in 10 ml of distilled water and serial dilution were made as 200, 100, 50, 25 and 10 µg ml^{-1} respectively and results were evaluated against test fungi.

3.3.4 Spore germination inhibition technique

All the test compounds were screened against four phytopathogenic fungi namely *P. grisea, D. oryzae, C. falcatum* and *U. hordei* by applying spore germination technique (Nene and Thapliyal 1993).

Spore suspension was made by adding sterilized distilled water to the fresh cultures of respective fungi. Suspension was filtered through three layers of sterilized cheese cloth in order to remove mycelial particles under aseptic conditions. Haemocytometer was used to standardize spore density (1 x 10^6 spores ml^{-1}). Small droplets (0.02 ml) of test solution and spore suspension in equal amount were seeded in the cavity of the cavity slides. These slides were placed in Petri plates lined with moist filter paper and were incubated for 24 hrs at 25±1 °C in case of *P. grisea, D. oryzae, C. falcatum* and *U. hordei.* In case of *U. hordei*, spore suspensions was made by adding distilled water to fresh water spores obtained from the infected breads of barley. The suspension filtered to remove the unwanted dust particles. The slides were incubated at 15±1 °C. The numbers of spores germinated were counted and per cent spore germination inhibition was calculated by the following formula:

$$I = \frac{C-T}{C} \times 100$$

Where, I = Per cent spore germination inhibition,

C = Spore germination in control and

T = Spore germination in treatment

3.3.5 Poisoned food technique

All the compounds were screened against *D. orzyae* by applying food poison technique (Grover and Moore, 1962). These were tested at different concentrations *viz.* 50, 100, 250, 500 and 1000 µg ml^{-1}. The culture of this fungus was obtained from maize section of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. These concentrations were further tested on PDA and the results were validated on the test fungus.

Preparation of culture media

Culture media is essential for isolation and sub-culturing. Potato Dextrose Agar media was used for maintaining them either in test tubes or in Petri plates.

Potato dextrose agar	
Potato dextrose broth (HiMedia)	24 g
Agar agar	24 g
Distilled water	1000 ml

Procedure for preparation of Potato dextrose agar (PDA)

Potato dextrose broth (24 g) was dissolved in 500 ml of distilled water. The solution was then boiled in a cooker for 20 minutes. After 20 minutes, 20 g of agar agar was added to that solution and boiled the mixture with continuous stirring until the agar agar was completely dissolved in the solution. Then total volume was made to one litre with distilled water. PDA solution of 99 ml was poured into sterilized conical flasks (250 ml) and were plugged with non absorbent cotton wool. The flasks were arranged, covered with a paper sheet and were sterilized in an autoclave at 15 psi (121 °C) for 20 minutes. The sterilized flasks were taken out after releasing the steam and were cooled at room temperature.

3.5.3 Poisoned food technique

A culture of the test fungi was grown in Petri plates on Potato Dextrose Agar (PDA) media and kept in incubator for seven days. Stock solution of test compounds was prepared in dimethyl sulfoxide at five concentrations (5, 10, 25, 50 and 100 mg ml^{-1}) and stored at 4 °C for further use. PDA supplemented with different prepared solutions at five concentrations (50, 100, 250, 500 and 1000 µg ml^{-1}) was poured in the Petri plates under aseptic conditions. After solidification, small disc (0.5 cm diameter) of the test fungi was cut with a sterile cork borer and transferred aseptically upside down at the center of Petri dish. Suitable checks were maintained, where the culture discs were grown under same conditions on PDA. Petri plates were incubated at 25 ± 1 °C and growth of fungus *D. orzyae* was recorded at regular intervals. Growth of test fungi colony was measured after every 24 hours till the fungus in the control plates completely occupied it. Each treatment was replicated thrice. The antifungal activity was evaluated by measuring the relative growth of fungus in each treatment (Grover and

Moore, 1962). The percent growth inhibition over control was worked out using the formula.

$$I = \frac{C-T}{C} \times 100$$

Where, I is inhibition percent,

C is colony diameter in control (cm) and

T is colony diameter in treatment (cm)

3.4 Statistical analysis

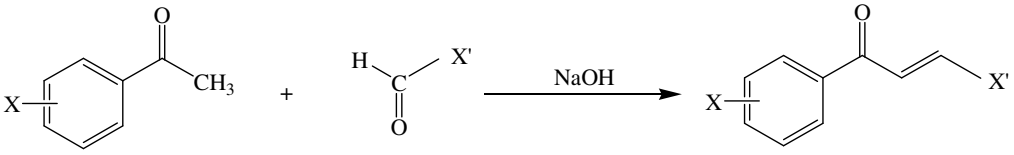
The significant difference in the antifungal activity of compounds was calculated using Complete Randomized Design (CRD).

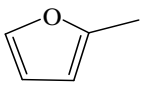
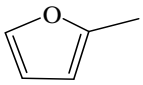
CHAPTER – IV

RESULTS AND DISCUSSION

4.1 Synthesis and characterization of halogenated chalcones

A solution of halogenated benzaldehyde (0.01 mole) dissolved in minimum quantity of methanol, was added drop wise to a stirred 10 ml methanolic solution of acetophenone (0.01 mole) containing 5 ml of 6 M NaOH solution. The solution was stirred at room temperature for another 20-30 minutes. The precipitates were filtered and dried in the folds of filter paper. The final product was recrystallized from chloroform to get halogenated chalcones (1-14). Physical data and elemental analysis of synthesized halogenated chalcones is enumerated in Table 1.



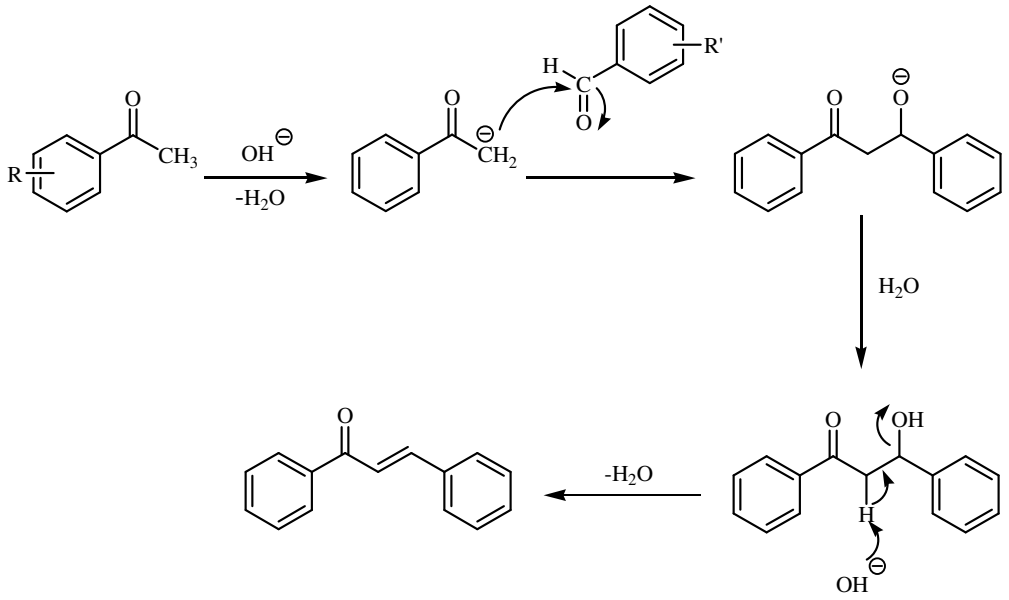
Compound	X	X'
1	H	4-Cl-Ph
2	4-Cl	4-Cl-Ph
3	H	4-F-Ph
4	4-F	4-F-Ph
5	4-F	3-Cl-Ph
6	4-F	2-Cl-Ph
7	4-F	4-Br-Ph
8	4-Br	3-Br-Ph
9	4-Br	2-Cl-Ph
10	4-Br	3-Cl-Ph
11	4-Br	4-F-Ph
12	4-Br	4-Br-Ph
13	4-F	
14	4-Br	

Scheme 1: General reaction of synthesis of chalcones

Mechanism

The chalcones were synthesized by reacting the appropriate acetophenones with halogenated benzaldehydes in the presence of catalytic amount of NaOH using methanol as solvent. This reaction is called Claisen-Schmidt condensation and the mechanistic detail of

the reaction with halogenated benzaldehyde and appropriate acetophenone as starting reagent is shown in Scheme 1. The methyl group of the acetophenone was deprotonated in the presence of base to give the enolate which attacked the electron deficient carbonyl carbon of the halogenated aldehyde following a nucleophillic addition reaction. The ensuing loss of water resulted in the halogenated chalcones.



Scheme 1: Mechanistic details of synthesis of chalcones

4.1.1 Charaterization of halogenated chalcones (1-14)

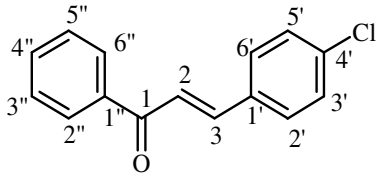
These were characterized by IR, ^{1}H NMR and ^{13}C NMR spectral analysis.

4.1.1.1 3-(4-Chlorophenyl)-1-phenylprop-2-en-1-one (1)

The IR spectrum of compound 1 showed a prominent band at 1664 cm^{-1} confirmed the C=O stretching and band at 1607 cm^{-1} confirmed the presence of C=C in conjugation with C=O stretching. Band at 736 cm^{-1} confirmed the C-Cl stretching.

^{1}H NMR of compound 1 showed two doublets between δ7.74-7.78 and δ8.01-8.03 due to protons of C_3 and C_2, respectively along with nine protons multiplet between δ7.26-7.62, that confirmed the presence of aromatic ring protons.

^{13}C NMR of the same compound showed the signal at 189.7 (C_1) due to carbonyl carbon, 121.8 (C_2) and 144.2 (C_3) signals are due to carbons of aliphatic double bond and signals at 129.9 ($C_{2''}$), 129.3 ($C_{3''}$), 133.6 ($C_{4''}$), 138.9 ($C_{1''}$), 126.9 ($C_{2'}$), 128.7 ($C_{3'}$) and 131.3 ($C_{1'}$) are due to aromatic carbons.



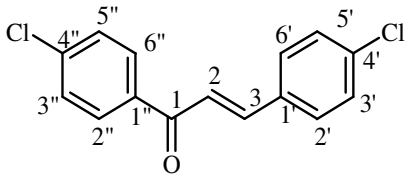
(1)

4.1.1.2 1, 3-Bis(4-chlorophenyl)prop-2-en-1-one (2)

In IR spectrum confirmed the formation of product by depicting a band at 1656 cm^{-1} due to C=O stretching and band at 1605 cm^{-1} confirmed the presence of C=C in conjugation with C=O stretching. Band at 726 cm^{-1} confirmed the C-Cl stretching.

^{1}H NMR of compound 2 showed two doublets between δ 7.94-7.96 and δ 8.20-8.25 due to one proton of $C_{3,}$ and one proton of $C_{2,}$ respectively. Eight protons multiplet between δ7.46-7.82 confirmed the protons of aromatic ring.

^{13}C NMR spectrum depicted signal at 189.7 (C_1) due to carbonyl carbon. The peaks at 121.6 (C_2) and 146.9 (C_3) confirmed the carbons of aliphatic double bond and peaks at 130.9 ($C_{2''}$), 128.3 ($C_{3''}$), 138.9 ($C_{4''}$), 138.6 ($C_{1''}$), 126.9 ($C_{2'}$), 128.7 ($C_{3'}$) and 131.3 ($C_{1'}$) are due to aromatic carbons.



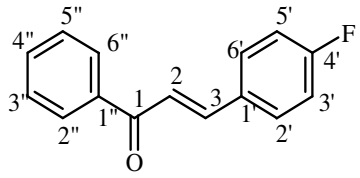
(2)

4.1.1.3 3-(4-Fluorophenyl)-1-phenylprop-2-en-1-one (3)

In IR spectrum, band at 1661 cm^{-1} observed due to C=O stretching. Another band at 1587 cm^{-1} is due to C=C which is present in conjugation with C=O stretching. . Band at 1156cm^{-1} confirmed the C-F stretching.

^{1}H NMR of compound 3 showed two doublets between δ7.77-7.99 and δ7.99-8.01 due to one proton of C_3 and C_2 proton, respectively. Nine protons multiplet between δ 6.95-7.73 confirmed the aromatic ring protons.

^{13}C NMR spectrum of the compound showed distinct signal at 191.6 (C_1) due to presence of carbonyl carbon. The peaks at 122.9 (C_2) and 143.3 (C_3) referred to the carbons of aliphatic double bond and peaks at 130.1 ($C_{2''}$), 128.7 ($C_{3''}$), 135.9 ($C_{4''}$), 138.5 ($C_{1''}$), 126.9 ($C_{2'}$), 120.7 ($C_{3'}$) and 130.4 ($C_{1'}$) are due to carbons of two rings.



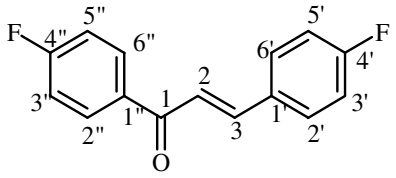
(3)

4.1.1.4 1, 3-Bis(4-fluorophenyl)prop-2-en-1-one (4).

IR spectrum showed band at 1666 cm^{-1} due to C=O stretching. At 1604 cm^{-1} band is observed due to presence of C=C in conjugation with C=O stretching. Band at 1161 cm^{-1} confirmed the C-F stretching

^{1}H NMR of compound 4 showed two doublets between δ7.78-7.81 and δ7.87-7.89 due to protons of C_3 and C_2, respectively. Eight protons multiplet between δ6.62-6.99 confirmed the aromatic ring protons.

The perusal of ^{13}C NMR for compound (4) showed peak at 188.8 (C_1) due to presence of carbonyl carbon. The peak at 120.6 (C_2) and 145.4 (C_3) corresponds to both carbons of aliphatic double bond. The peaks at 130.8 ($C_{2''}$), 119.7 ($C_{3''}$), 150.9 ($C_{4''}$), 135.5 ($C_{1''}$), 126.9 ($C_{2'}$), 118.7 ($C_{3'}$) and 130.4 ($C_{1'}$) are observed due to aromatic carbons.



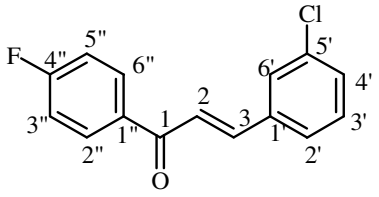
(4)

4.1.1.5 3-(3-Chlorophenyl)-1-(4-fluorophenyl) prop-2-en-1-one (5)

Band at 1668 cm^{-1} observed due to C=O stretching and due to C=C stretching band at 1609 cm^{-1} was registered in IR spectrum. Band at 685 cm^{-1} confirmed the C-Cl stretching and 1158 cm^{-1} confirmed C-F stretching.

^{1}H NMR of compound 5 showed two doublets between δ7.73-7.77 and δ7.99-8.01 due to one proton of C_3 and C_2 proton, respectively. Eight protons multiplet between δ7.05-7.66 confirmed the aromatic ring protons.

The signal at 189.4 (C_1) in the ^{13}C NMR spectrum was due to carbon of carbonyl group. The carbons of double bond of chain represented by carbon C_2 and C_3 appeared at 121.9 and 144.6 respectively. The signal due to aromatic carbons occurred at 130.8 ($C_{2''}$), 119.7 ($C_{3''}$), 157.9 ($C_{4''}$), 134.6 ($C_{1''}$), 122.9 ($C_{2'}$), 130.7 ($C_{3'}$) and 135.9 ($C_{1'}$).



(5)

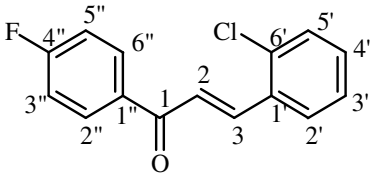
4.1.1.6 3-(2-Chlorophenyl)-1-(4-fluorophenyl)prop-2-en-1-one (6)

Band at 1662 cm^{-1} observed due to C=O stretching and due to C=C stretching band at 1603 cm^{-1} was registered in IR spectrum. Band at 705 cm^{-1} confirmed the C-Cl stretching and 1156 cm^{-1} confirmed C-F stretching.

^{1}H NMR of compound 6 showed two doublets between δ7.73-7.77 and δ7.99-8.01 due to protons of C_3 and C_2, respectively along with eight protons multiplet between δ7.05-7.66 confirmed the aromatic ring protons.

^{13}C NMR of the same compound showed the signal at 188.8 (C_1) due to carbonyl

carbon, 124.4 (C_2) and 140.8 (C_3) signal are due to carbons of double bond and signals at 130.3 ($C_{2''}$), 115.7 ($C_{3''}$), 164.4 ($C_{4''}$), 134.3 ($C_{1''}$), 127.0 ($C_{2'}$), 131.2 ($C_{3'}$) and 135.5 ($C_{1'}$) are due to aromatic carbons.



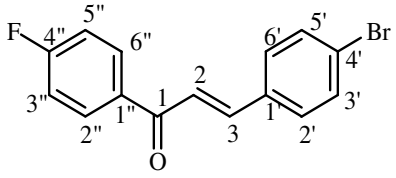
(6)

4.1.1.7 1-(4-Bromophenyl)-3-(4-fluorophenyl) prop-2-en-1-one (7)

IR spectrum showed band at 1663 cm^{-1} due to C=O stretching. At 1606 cm^{-1} band is observed due to presence of C=C in conjugation with C=O stretching. Band at 665 cm^{-1} confirmed the C-Br stretching and 1159 cm^{-1} confirmed C-F stretching.

^{1}H NMR of compound 7 showed two doublets between δ7.73-7.77 and δ8.06-8.09 due to one proton of C_3 and C_2 proton, respectively. Eight protons multiplet between δ7.38-7.62 confirmed the aromatic ring protons.

The signal due to carbon carbonyl group is at 185.4 (C_1) in the ^{13}C NMR spectrum. The carbons of double bond of chain represented by carbon C_2 and C_3 appeared at 121.9 and 141.7 respectively. The signal due to aromatic carbons occurred at 130.8 ($C_{2''}$), 117.7 ($C_{3''}$), 161.9 ($C_{4''}$), 132.6 ($C_{1''}$), 127.9 ($C_{2'}$), 131.3 ($C_{3'}$) and 135.1 ($C_{1'}$).



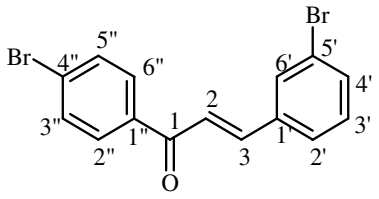
(7)

4.1.1.8 3-(3-Bromophenyl)-1-(4-bromophenyl) prop-2-en-1-one (8)

In IR spectrum, band at 1664 cm^{-1} observed due to C=O stretching. Another band at 1607 cm^{-1} is due to C=C which is present in conjugation with C=O stretching. Band at 661 cm^{-1} confirmed the C-Br stretching.

^{1}H NMR of compound 8 showed two doublets between δ7.27-7.30 and δ7.46-7.49 due to one proton of C_3 and C_2 proton, respectively. Eight protons multiplet between δ7.01-7.21 confirmed the aromatic ring protons.

^{13}C NMR of the same compound showed the signal at 188.89 (C_1) due to carbonyl carbon, 122.6 (C_2) and 143.5 (C_3) signal are due to carbons of double bond and signals at 133.4 ($C_{2''}$), 132.0 ($C_{3''}$), 128.2 ($C_{4''}$), 136.6 ($C_{1''}$), 123.1 ($C_{2'}$), 130.8 ($C_{3'}$) and 136.6 ($C_{1'}$) are due to aromatic carbons.



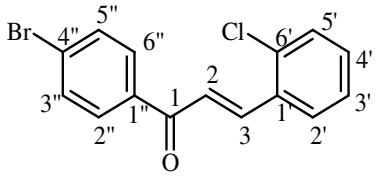
(8)

4.1.1.9 1-(4-Bromophenyl)-3-(2-chlorophenyl)prop-2-en-1-one (9)

The IR spectrum of compound 9 showed a prominent band at 1665 cm^{-1} confirmed the C=O stretching and band at 1607 cm^{-1} confirmed the presence of C=C in conjugation with C=O stretching. Band at 678 cm^{-1} confirmed the C-Br stretching and 727 cm^{-1} confirmed C-Cl stretching.

^{1}H NMR of compound 9 showed two doublets between δ7.21-7.27 and δ7.37-7.40 due to protons of C_3 and C_2, respectively along with eight protons multiplet between δ6.66-7.10 confirmed the aromatic ring protons.

^{13}C NMR of the same compound showed the signal at 189.34 (C_1) due to carbonyl carbon, 124.2 (C_2) and 141.1 (C_3) signal are due to carbons of double bond and signals at 131.3 ($C_{2''}$), 131.9 ($C_{3''}$), 128.1 ($C_{4''}$), 136.6 ($C_{1''}$), 127.8 ($C_{2'}$), 127.1 ($C_{3'}$) and 133.0 ($C_{1'}$) are due to aromatic carbons.



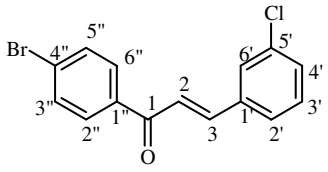
(9)

4.1.1.10 1-(4-Bromophenyl)-3-(3-chlorophenyl) prop-2-en-1-one (10)

The IR spectrum of compound 10 showed a prominent band at 1662 cm^{-1} confirmed the C=O stretching and band at 1601 cm^{-1} confirmed the presence of C=C in conjugation with C=O stretching. Band at 660 cm^{-1} confirmed the C-Br stretching and 712 cm^{-1} confirmed C-Cl stretching.

^{1}H NMR of compound 10 showed two doublets between δ7.51-7.57 and δ7.78-7.82 due to one proton of C_3 and C_2 proton, respectively. Eight protons multiplet between δ7.01-7.44 confirmed the aromatic ring protons.

^{13}C NMR of the same compound showed the signal at 189.5 (C_1) due to carbonyl carbon, 123.7 (C_2) and 146.3 (C_3) signal are due to carbons of double bond and signals at 131.5 ($C_{2''}$), 133.0 ($C_{3''}$), 128.7 ($C_{4''}$), 135.7 ($C_{1''}$), 125.1 ($C_{2'}$), 130.3 ($C_{3'}$) and 136.1 ($C_{1'}$) are due to aromatic carbons.



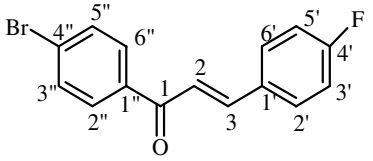
(10)

4.1.1.11 3-(4-Bromophenyl)-1-(4-fluorophenyl) prop-2-en-1-one (11)

The IR spectrum of compound 11 showed a prominent band at 1667 cm^{-1} confirmed the C=O stretching and band at 1604 cm^{-1} confirmed the presence of C=C in conjugation with C=O stretching. Band at 660 cm^{-1} confirmed the C-Br stretching and 1157 cm^{-1} confirmed C-F stretching.

^{1}H NMR of compound 11 showed two doublets between δ7.66-7.69 and δ8.07-8.11 due to protons of C_3 and C_2, respectively. Eight protons multiplet between δ7.21-7.48 confirmed the aromatic ring protons..

^{13}C NMR of the same compound showed the signal at 189.5 (C_1) due to carbonyl carbon, 121.4 (C_2) and 144.2 (C_3) signal are due to carbons of double bond and signals at 131.8 ($C_{2''}$), 132.5 ($C_{3''}$), 128.5 ($C_{4''}$), 135.9 ($C_{1''}$), 127.9 ($C_{2'}$), 117.3 ($C_{3'}$) and 131.1 ($C_{1'}$) are due to aromatic carbons.



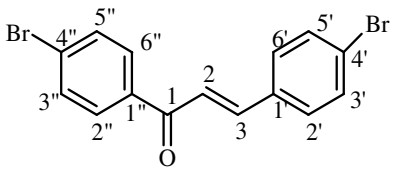
(11)

4.1.1.12 1, 3-Bis(4-bromophenyl)prop-2-en-1-one (12)

The IR spectrum of compound 12 showed a prominent band at 1656 cm^{-1} confirmed the C=O stretching and band at 1603 cm^{-1} confirmed the presence of C=C in conjugation with C=O stretching. Band at 663 cm^{-1} confirmed the C-Br stretching.

In compound 12, ^{1}H NMR showed two doublets between δ7.52- 7.56 and δ7.76-7.79 due to one proton of C_3 and C_2 proton, respectively along with eight protons multiplet between δ7.21- 7.25 confirmed the protons of aromatic ring.

The signal at 189.4 (C_1) in the ^{13}C NMR spectrum was due to carbon of carbonyl group. The carbons of double bond of chain represented by carbon C_2 and C_3 appeared at 121.9 and 144.6 respectively. The signal due to aromatic carbons occurred at 130.8 ($C_{2''}$), 119.7 ($C_{3''}$), 157.9 ($C_{4''}$), 134.6 ($C_{1''}$), 122.9 ($C_{2'}$), 130.7 ($C_{3'}$) and 135.9 ($C_{1'}$).



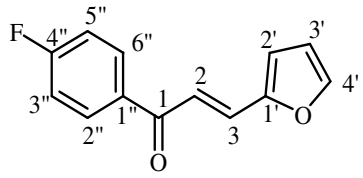
(12)

41.1.13 1-(4-Fluorophenyl)-3-(furan-2-yl) prop-2-en-1-one (13)

The IR spectrum of compound 13 showed a prominent band at 1663 cm^{-1} confirmed the C=O stretching and band at 1603 cm^{-1} confirmed the presence of C=C in conjugation with C=O stretching. Band at 1161 cm^{-1} confirmed C-F stretching.

^{1}H NMR of compound 13 showed two doublets between δ7.58-7.61 and δ7.80-7.88 due to one proton of C_3 and C_2 proton, respectively. Eight protons multiplet between δ6.64-6.73 confirmed the aromatic ring protons.

The perusal of ^{13}C NMR for compound 13 showed peak at 188.8 (C_1) due to presence of carbonyl carbon. The peak at 120.6 (C_2) and 145.4 (C_3) corresponds to both carbons of aliphatic double bond. The peaks at 130.8 ($C_{2''}$), 119.7 ($C_{3''}$), 150.9 ($C_{4''}$), 135.5 ($C_{1''}$), 126.9 ($C_{2'}$), 118.7 ($C_{3'}$) and 130.4 ($C_{1'}$) are observed due to aromatic carbons.



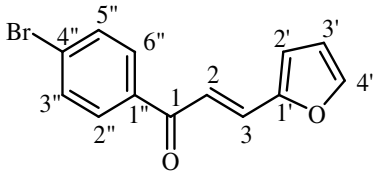
(13)

4.1.1.14 1-(4-Bromophenyl)-3-(furan-2-yl) prop-2-en-1-one (14)

The IR spectrum of compound 14 showed a prominent band at 1655 cm^{-1} confirmed the C=O stretching and band at 1601 cm^{-1} confirmed the presence of C=C in conjugation with C=O stretching. Band at 662 cm^{-1} confirmed the C-Br stretching.

^{1}H NMR of compound 14 showed two doublets between δ6.51-6.52 and δ6.73-6.74 due to one proton of C_3 and C_2 proton, respectively. Seven protons multiplet between δ7.26-7.90 confirmed the aromatic protons.

^{13}C NMR of the same compound showed the signal at 188.6 (C_1) due to carbonyl carbon, 118.6 (C_2) and 145.1 (C_3) signal are due to carbons of double bond and signals at 129.9 ($C_{2''}$), 166.6 ($C_{3''}$), 151.5 ($C_{4''}$), 136.9 ($C_{1''}$), 127.8 ($C_{2'}$), 115.8 ($C_{3'}$) and 131.1 ($C_{1'}$) are observed due to aromatic carbons.



(14)

The present research revealed an easy, clean and efficient method for the synthesis of different substituted halogenated chalcones. The products were obtained in good yields, short reaction time and were stable at room temperature. For the synthesis of halogenated chalcones, this method was found to be as effective as microwave irradiation method than the conventional heating techniques (Srivastava 2008).

Table 1: Elemental analysis and physical properties of synthesized halogenated chalcones (1-14)

Compound No.	State	Color/λ_{max}(nm)	Yield (%)	Melting point (°C)	Elemental analysis calculated (found) %			Molecular Formula
					C	H	O	
1	Solid	Light Yellow/351	88	165	74.23(74.22)	4.57(4.57)	6.59(6.57)	$C_{15}H_{11}OCl$
2	Solid	Pale Yellow/363	81	155	65.01(65.00)	3.64(3.66)	5.77(5.07)	$C_{15}H_{10}OCl_2$
3	Solid	Cream/363	83	155	79.63(79.59)	4.90(4.96)	7.07(7.07)	$C_{15}H_{11}OF$
4	Solid	Pale Yellow/358	85	117	73.76(70.76)	4.13(4.88)	6.55(6.55)	$C_{15}H_{10}OF_2$
5	Solid	Yellow/353	82	105	69.11(69.11)	3.87(3.88)	6.14(6.70)	$C_{15}H_{10}OClF$
6	Solid	Yellow/361	77	85	69.11(70.05)	3.87(3.61)	6.14(6.14)	$C_{15}H_{10}OClF$
7	Solid	Pale Yellow/360	86	105	59.04(60.00)	3.30(3.61)	5.24(5.24)	$C_{15}H_{10}OFBr$
8	Solid	Yellow/352	89	122	49.22(49.21)	2.75(2.73)	4.37(4.37)	$C_{15}H_{10}OBr_2$
9	Solid	Yellow/358	87	137	56.02(57.11)	3.13(3.13)	4.97(4.98)	$C_{15}H_{10}OBrCl$
10	Solid	Pale Yellow/363	80	122	56.02(56.02)	3.13(3.10)	4.97(4.23)	$C_{15}H_{10}OBrCl$
11	Solid	Light Yellow/352	69	160	59.04(56.02)	3.30(3.10)	5.24(4.24)	$C_{15}H_{10}OFBr$
12	Solid	Yellow/361	78	122	49.22(50.11)	2.75(2.70)	4.37(4.24)	$C_{15}H_{10}OBr_2$
13	Solid	Light Brown/360	74	140	72.22(72.22)	4.20(4.03)	14.80(14.84)	$C_{13}H_9O_2F$
14	Solid	Dark Brown/357	81	102	56.34(56.34)	3.27(4.12)	11.55(11.55)	$C_{13}H_9O_2Br$

Nano emulsions of Halogenated Chalcones

Halogenated chalcones demonstrated limited solubility in common organic solvents. However, in the present study oleic acid was selected as the best oil phase by virtue of its maximum halogenated chalcone solubility with heating for a very short time, compared to castor oil and groundnut oil that have high density and form a non-transparent solution above 20 mg ml^{-1} halogented chalcone concentration. Tween 20 was considered as good surfactant because it lowered the necessary energy to form nano emulsion that consequently improved the solubility and have high miscibility with oil phase. The addition of co-surfactant acetone enhanced the stability of the nano emulsion by reducing the droplet size, which may be due to the rapid diffusion of the co-surfactant by the evaporation. The nano emulsion thus formed was transparent.

The nano emulsion droplet when observed under TEM appeared dark and the surrounding was bright. The nano emulsion formation was discrete, spherical, rod and smooth (Fig 1). The size of these oil globules ranged from 50 nm to 100 nm.

4.2 Antifungal activity of synthesized halogenated chalcones

4.2.1 Activity using spore germination technique

The synthesized Halogenated Chalcones were screened *in vitro* against *Dreschslera oryzae*, *Ustilago hordei*, *Pyricularia grisea* and *Colletotrichum falcatum* by spore germination inhibition technique. The effective dose is expressed in terms of ED_{50} values at which 50 per cent inhibition has occurred. The data was the mean of three replicate tests performed for each antifungal test, calculated using descriptive analysis option in SPSS. All the compounds were tested against four fungi.

4.2.1.1 *D. oryzae*

The phytopathogenic activity of all the compounds against *D. oryzae* was mild having ED_{50} values less than 245µg ml^{-1}. The inhibition percentage values at different concentration are given in Table 2. Compound 13 and 14 showed least ED_{50} value at 180 and 185 µg ml^{-1} respectively. It shows that compounds 13 and 14 were more effective than other synthesized halogenated compounds. So out of all synthesized compounds, compound 13 and 14 having heterocyclic moiety bearing flouro and bromo group respectively showed maximum inhibition of test fungi (Varo *et al* 2014). Compound 3 having one flouro group showed maximum ED_{50} value *i.e* least effective compound followed by compound 1 having one chloro group. Dihalogenated compound 8 and 12 bearing bromo groups also have good ED_{50} value of 195 µg ml^{-1}. Compounds containing chloro group are less effective than bromo bearing compounds but are more effective than compounds having flouro group (Hasan *et al* 2017). But none of the compound was found as effective as standard fungicide propicanozole 25 EC (ED_{50} = 22 µg ml^{-1}).

4.2.1.2 *U. hordei*

The inhibition per cent values of all the synthesized compounds against *U. hordei* given in Table 3 were found to be high at higher concentrations (1000, 500 and 250 μg ml^{-1}) but moderate at lower concentrations (100 and 50 μg ml^{-1}). All synthesized halogenated compounds have ED_{50} value of less than 255 μg ml^{-1}. Dihalogenated compound 8 and compound 12 having two bromo groups attached to ring showed the least ED_{50} value and hence more effective among all the synthesized halogenated chalcones (Hasan *et al* 2017). Compound 3 had ED_{50} value 255 μg mL^{-1} having one flouro atom and was least effective. Compound 13 and 14 also showed activity against *U. hordei* with ED_{50} values of 190 μg ml^{-1} and 195 μg ml^{-1} respectively. The effectiveness of compound 13 and 14 was due to heterocyclic moiety (Seelam *et al* 2013). None of the compound was found as effective as standard fungicide Tebuconazole (Raxil) (ED_{50} = 25 μg ml^{-1}).

4.2.1.3 *P. grisea*

The data presented in Table 4 exhibited that all the synthesized halogenated chalcones had less activity than Standard at all the concentrations. Compound 13 having bromo group and heterocyclic ring showed maximum inhibition per cent with ED_{50} 190 μg ml^{-1} (Varo *et al* 2014). Dihalogented compounds *i.e* 8 and 11 bearing two bromo groups also show inhibition per cent against this fungus having ED_{50} value of 200 μg ml^{-1} (Hasan *et al* 2017). All the compounds show ED_{50} value less than 240 μg ml^{-1}. None of the synthesized compound was found to be as effective as standard fungicide propiconazole 25 EC (ED_{50} = 15 μg ml^{-1}).

4.2.1.4 *C. falcatum*

Against *C. falcatum* all the synthesized compounds showed mild results with $ED_{50} \leq$ 245 μg ml^{-1}. The inhibition percentage values of all the test compounds are given in Table 5. The compounds 13 and 14 showed maximum inhibition per cent with ED_{50} value 180 and 195 μg ml^{-1} respectively. Dihalogenated compounds bearing two bromo groups had ED_{50} values 200 μg ml^{-1} (Seelam *et al* 2013). All other compounds also showed mild inhibition against test fungi but all are less effective than the standard fungicide Carbendazim 50 WP (ED_{50} = 31 μg ml^{-1}).

Table 2: Effect of different concentrations of halogenated chalcones on per cent inhibition against spores of *D. orzyae*

Compound No.	Per cent inhibition of spores at different concentrations (μg ml^{-1})				
	1000	**500**	**250**	**100**	**50**
1	87.33±0.65	70.94±0.82	51.95±0.65	22.21±0.57	0.00±0.95
2	89.65±0.55	74.00±0.65	58.45±0.89	24.55±0.55	6.94±0.05
3	86.44±0.41	69.55±0.85	51.55±0.85	20.64±0.88	0.00±0.55
4	87.55±0.65	68.54±0.95	52.00±0.54	30.55±0.85	3.15±0.79
5	87.94±0.30	71.55±0.55	54.25±0.75	30.95±0.77	5.25±0.75
6	88.05±0.75	71.05±0.65	55.05±0.91	31.66±0.88	5.15±0.79
7	96.65±0.66	74.21±0.78	61.85±0.85	27.46±0.55	7.05±0.78
8	92.00±0.94	78.55±0.85	60.00±0.95	25.64±0.99	5.00±0.65
9	97.00±0.75	75.15±0.65	62.00±0.85	30.22±0.75	7.75±0.74
10	97.65±0.85	78.65±0.85	62.45±0.55	29.51±0.88	7.15±0.44
11	88.75±0.81	72.45±0.75	55.68±0.77	33.45±0.76	6.31±0.64
12	98.44±0.56	80.00±0.88	60.56±0.87	25.54±0.79	5.00±0.88
13	100.00±0.85	81.25±0.85	62.00±0.59	30.22±0.99	7.75±0.95
14	100.00±0.95	80.55±0.95	61.85±0.57	27.46±0.95	7.05±0.85
*** Propiconazole (Tilt)**	100.00±0.00	100.00±0.00	95.00±0.46	88.00±0.79	65.00±0.29

*Standard fungicide for *D. orzyae*

Table 3: Effect of different concentrations of halogenated chalcones on per cent inhibition against spores of *U.hordei*

Compound No.	Per cent inhibition of spores at different concentrations (µg ml^{-1})				
	1000	**500**	**250**	**100**	**50**
1	82.66±0.86	66.45±0.73	50.21±0.73	24.65±0.77	1.25±0.66
2	88.65±0.70	71.54±0.77	55.66±0.68	26.55±0.54	7.54±0.47
3	81.25±0.78	65.25±0.80	49.85±0.56	23.55±0.92	0.00±0.38
4	84.66±0.68	67.85±0.76	51.51±0.90	25.12±0.88	4.25±0.96
5	85.47±0.54	69.44±0.83	52.45±0.86	27.33±0.64	5.51±0.48
6	85.00±0.45	70.12 ±0.79	52.15±0.72	27.05±0.76	5.15±0.73
7	89.45±0.67	73.45±0.48	55.98±0.77	28.65±0.88	9.65±0.78
8	94.75±0.84	81.62±0.91	60.00±0.48	29.05±0.93	18.43±0.5
9	90.54±0.74	78.54±0.57	62.45±0.45	31.44±0.66	10.54±0.63
10	91.45±0.69	77.45±0.59	62.05±0.55	29.45±0.82	11.75±0.58
11	86.24±0.58	70.21±0.68	54.22±0.66	26.55±0.44	6.52±0.71
12	93.45±0.60	80.74±0.75	60.14±0.70	29.81±0.58	15.74±0.46
13	91.75±0.67	79.71±0.62	61.75±0.80	30.14±0.71	7.75±0.59
14	92.75±0.77	80.43±0.71	60.75±0.87	27.45±0.86	13.75±0.67
**** Tebuconazole (Raxil)**	100.00±0.00	100.00±0.00	87.00±0.03	77.50±0.87	59.62±0.45

**Standard fungicide for *U.hordei*

Table 4: Effect of different concentrations of halogenated chalcones on per cent inhibition against spores of *P. grisea*

Compound No.	Per cent inhibition of spores at different concentrations (µg ml^{-1})				
	1000	**500**	**250**	**100**	**50**
1	82.66±0.65	66.45±0.68	50.21±0.67	24.65±0.46	2.47±0.79
2	87.79±0.54	73.67±0.49	54.87±0.98	27.90±0.89	7.48±0.86
3	83.74±0.39	66.24±0.86	51.42±0.34	24.74±0.43	1.45±0.58
4	84.74±0.61	67.95±0.59	51.94±0.20	25.48±0.62	4.63±0.68
5	86.44±0.43	70.96±0.61	51.99±0.60	28.05±0.71	5.05±0.54
6	86.75±0.73	70.14±0.90	52.61±0.85	27.63±0.57	5.97±0.53
7	88.67±0.55	74.82±0.86	55.99±0.56	29.87±0.71	9.61±0.59
8	91.45±0.95	77.82±0.60	58.10±0.76	31.98±0.69	13.75±0.61
9	91.45±0.69	79.64±0.65	63.40±0.46	31.55±0.58	10.66±0.68
10	90.51±0.78	78.40±0.73	62.10±0.79	30.47±0.50	11.00±0.74
11	86.86±0.67	71.84±0.49	53.78±0.45	26.48±0.84	6.52±0.71
12	90.84±0.44	75.84±0.77	57.00±0.63	31.70±0.62	12.87±0.56
13	92.84±0.86	79.50±0.83	58.47±0.55	30.80±0.56	13.84±0.47
14	91.00±0.62	78.40±0.59	57.90±0.45	29.04±0.74	12.00±0.89
*** Propiconazole (Tilt)**	100.00±0.36	100.00±0.28	95.05±0.26	88.41±0.41	75.54±0.04

*Standard fungicide for *P. grisea*

Table 5: Effect of different concentrations of halogenated chalcones on per cent inhibition against spores of *C. falcatum*

Compound No.	Per cent inhibition of spores at different concentrations (μg ml^{-1})				
	1000	**500**	**250**	**100**	**50**
1	82.12±0.53	69.88±0.64	51.20±0.78	24.60±0.64	1.47±0.77
2	86.45±0.83	74.10±0.48	55.10±0.58	28.90±0.83	4.80±0.61
3	81.45±0.74	68.47±0.48	51.00±0.85	23.41±0.58	0.00±0.82
4	82.40±0.91	69.80±0.39	52.11±0.54	26.00±0.55	1.58±0.37
5	84.98±0.85	70.00±0.46	53.78±0.49	27.12±0.69	2.45±0.44
6	85.10±0.89	70.14±0.76	53.40±0.67	27.00±0.53	3.11±0.99
7	88.67±0.48	74.20±0.72	56.12±0.93	29.10±0.51	6.780±0.73
8	88.40±0.32	77.50±0.57	58.46±0.46	30.80±0.42	13.75±0.94
9	88.00±0.51	76.36±0.16	57.60±0.36	29.15±0.68	6.88±0.59
10	88.12±0.40	76.58±0.57	57.12±0.73	29.40±0.81	9.10±0.82
11	85.41±0.64	71.84±0.63	55.00±0.62	27.84±0.66	3.90±0.74
12	89.41±0.38	75.40±0.38	58.70±0.88	30.15±0.41	10.00±0.88
13	90.15±0.57	77.66±0.72	59.44±0.47	32.41±0.72	13.50±0.95
14	90.05±0.87	77.54±0.59	59.00±0.72	31.44±0.63	10.87±0.42
***** Carbendazm (Bavistin)**	100.00±0.00	92.45±0.62	81.96±0.94	76.54±0.61	60.00±0.15

***Standard fungicide for *C. falcatum*

Table 6: ED_{50} values of synthesized halogenated compounds against *D. oryzae, U. hordei, P. grisea* and *C. falcatum*

Compound	*D. oryzae*	*U. hordei*	*P. grisea*	*C. falcatum*
1	240	245	240	235
2	205	215	220	220
3	245	255	240	245
4	230	240	235	235
5	215	235	230	225
6	210	230	230	220
7	205	205	210	210
8	195	185	200	200
9	200	210	205	205
10	205	200	200	210
11	210	225	225	215
12	195	185	200	200
13	180	190	190	180
14	185	195	195	195
Propiconazole (Tilt)	22	*	15	*
Tebuconazole (Raxil)	*	25	*	*
Carbendazm (Bavistin)	*	*	*	31
CD (p = 0.05)	1.67	1.67	1.67	1.67

4.2.2 Antifungal activity of compounds using poisoned food technique

4.3.1 *D. orzyae*

The test solutions were evaluated for their fungitoxicity by this technique at different concentrations *viz.* 1000, 500, 250, 100 and 50 µg ml^{-1}. The results were expressed in terms of percentage radial growth inhibition, from which ED_{50} values were calculated using polo software programme (Table 7). Tilt was used as standard against the test fungus.

The ED_{50} values in table clearly implicates that all the halogenated compounds against *D. orzyae* showed moderate results. The compounds 13 and 14 showed good result against this test fungus with minimum ED_{50} values of 182 and 188 respectively. Compounds having bromo group also showed good results against *D. orzyae*. But none of the synthesized halogenated compound was as effective as the standard fungicide. All the treatments were significantly different from each other at all concentrations.

Table 7: ED_{50} values of different halogenated compounds against *D. orzyae*

Compound No.	ED_{50} (µg ml^{-1})
1	243
2	206
3	245
4	234
5	216
6	210
7	208
8	195
9	203
10	204
11	211
12	196
13	182
14	188
Tilt	22

There is very limited reported literature on the chalcones to address agriculture related problems (Hasan *et al* 2007) and comparison of the results with the present work. Overall, the results against the test fungi revealed mild activities of the synthesized chalcones in comparison to the commercial standards used. The results were in comparison to the microbiological assays against various bacteria (MIC=250) (Ritter *et al* 2015). The chalcones are analogous of natural products and they were applied as the water based nanoformulations, which is eco-friendly side of the chemical applications. Overall, the chalcones can be the

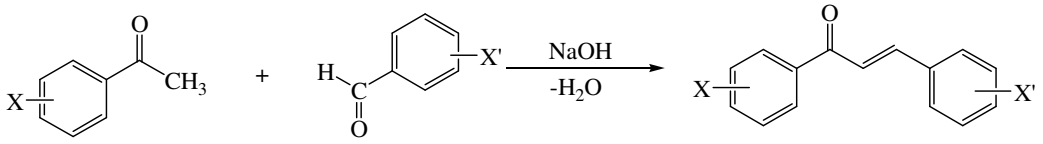
potential candidates even at this concentrations (≈200 μg ml^{-1}) for the further exploration after studying their potential health effects on humans and other micro flora.

CHAPTER V

SUMMARY

Chalcones contain two aromatic rings that are linked by an aliphatic three carbon chain. They are considered to be a standout amongst the most interesting and challenging area of chemistry embracing a wide spectrum of advances of both practical and hypothetical significance. One interesting trend that was observed in the literature is the association of halogens atoms on the chalcone template with a particular activity profile. Halogen present on chalcone exerted a strong influence on the physiochemical profile of the molecule. Recently it was found that α-bromo chalcones had higher physiological activities and it is well-known that introducing fluorine into some molecules is a powerful strategy to optimize the properties of pharmaceutical and agricultural products (Leroux 2016).

The objective for the synthesis of halogenated chalcones was that the presence of halogen atom on chalcone was expected to enhance the antifungal activity. To this end, a series of chalcones bearing halogen atom were synthesized by the base catalyzed Claisen-Schmidt condensation and evaluated for the antifungal activity against *Dreschslera oryzae*, *Ustilago hordei*, *Pyricularia grisea* and *Colletotrichum falcatum*.



Physical data (yield, melting point, state and color) of synthesized halogenated compounds was determined. The synthesized compounds were characterized on the basis of IR, ^{1}H NMR and ^{13}C NMR spectroscopy techniques and their antifungal efficacy were evaluated by spore germination inhibition technique. The results indicated that most of the compounds had moderate fungi toxicity against the test fungi with ED_{50} values of all the test compounds less than 255 μg ml^{-1}.

Synthesized compounds were found to inflict mild activity against *D. orzyae* and *U. hordei*. Heterocyclic analogues (13 and 14) having halogen atom on ring showed maximum inhibition percent with ED_{50} values 180 and 185 μg ml^{-1} respectively against *D. orzyae* .The antifungal potential of the test compounds against *P. grisea* and *C. falcatum* showed the same results as against *D. orzyae*. Heterocyclic compounds having halogens were found to be most effective among all the synthesized halogenated chalcones. Since, they are analogues of natural bioactive motif of plant kingdom and were applied as eco-friendly water based formulations, they can be considered for further research and exploration after proper studies on humans and other micro flora.

CONCLUSION

The halogenated chalcones were synthesized by reacting different aldehydes with different α,β unsaturated ketones in presence of catalytic amount of sodium hydroxide by conventional method. Characterization of the synthesized compounds was done by IR, ^{1}H NMR and ^{13}C NMR. The synthesized halogenated chalcones were also screened for their antifungal potential against *Dreschslera oryzae*, *Ustilago hordei*, *Pyricularia grisea* and *Colletotrichum falcatum*. All the synthesized compounds were having mild results against all the test fungi but compound 13 and 14 showed better results than the other compounds. But none of the synthesized halogenated compound was as effective as standard.

REFERENCES

Alarcon E, Romero N, Aguilar H, Teran J L, Gomez L, Roa L F, Lobato C E and Escobar A (2013) Green synthesis of chalcone derivatives of acetophenone. *Green Chem* **5**: 26-69.

Albogami A S, Karama U, Mousa A A, Khan M, Al-Mazroa S A and Alkhathlan H Z (2012) Simple and efficient one step synthesis of functionalized flavanones and chalcones. *Oreintal J Chem* **28**(2): 619-26.

Aly M, Fodah H and Saleh S Y (2014) Antioxidant and cytotoxicity activities of newly synthesized chalcone derivatives and their metal complexes. *Eur J Med Chem* **76**:517-30.

Ameta K L, Rathore N S and Kumar B (2011) Synthesis of some novel chalcones and their facile one-pot conversion to 2-aminobenzene-1,3-dicarbonitriles using malononitrile. *Analele Universităţii din Bucureşti* **20**(1): 15-24.

Amit B, Lokesh C M, Manish S, Satish K A and Virendra K B (2009) Synthesis of various chalcones and their antimalarial activity. *Eur J Med Chem* **44**: 33-88.

Arora V, Arora P and Lamba H S (2012) Synthesis and evaluation of chalcone derivatives of 2-acetyl-napthalene for antifungal and antibacterial activity. *Der Pharmica Lettre* **4**(2): 554-57.

Asiri A M and Khan S A (2011) Synthesis and anti-bacterial activities of a bis-chalcone derived from thiophene and its bis-cyclized products. *Molecules* **16**: 523-31.

Babasaheb P B, Sachin A P, Balaji L K, Shivraj H N and Chandrahase N K (2011) Synthesis and characterization of some chalcone derivatives. *Eur J Med* Chem **45**: 26-9.

Bohui L, Xue X, Xia W, Hua Y, Xiuxiu L, Weiyang T, Yonghua W and Ling Y (2011) Method for extracting active ingredients from Chinese medicine Dalbergia and their application as cardiovascular agent. CN102119957.

Bonesi M, Loizzo M R, Statti G A, Michel S, Tillequin F and Menichini F (2010) The synthesis and angiotensin converting Enzyme (ACE) inhibitory activity of chalcones and their pyrazole derivatives. *Bioorg Med Chem* **20**: 1990-93.

Borate H B (2011) Hybrid molecules containing pharmacophores of fluconazole as antifungal agents and their preparation. INMU01750.

Bozic D D, Milenkovic, Ivkovic B, Cirkovic I (2014) Antibacterial activity of three newly-synthesized chalcones and synergism with antibiotics against clinical isolates of methicillin-resistant *Staphylococcus aureus. Ind J Med Res* **140**: 130-37

Chate A V, Joshi R S, Mandhane P G and Gill C H (2012) Synthesis, characterization and antimicrobial screening of some novel chalcones and their derivatives. *Ind J Chem* **51B**: 1642-48.

Chavan B B, Gadekar A S, Mehta P P, Vawhal P K, Kolsure A K and Chabukswar A R (2016) Synthesis and medicinal significance of chalcones: A review. *Asian J Biomed Pharma* **56** (6): 1-7.

Clayden J, Greeves N, Warren S and Wothers P (2007) *Organic Chemistry*. Oxford Press, New York.

Concellon J M and Huerta M (2002) Preparation of (Z)-□-chloro-□,□-unsaturated ketones with total or high diastereoselectivity. *Tetrahedron* **58**: 7775-80.

Dhananjayulu M, Prasad M S, Swarupa C, Naik M S and Sreedhar N Y (2014) Synthesis, characterization and electrochemical behaviour of 2-hydroxy chalcones using silica gel supported piperidine. *IJIRSET* **3**(5): 12496-501.

Doan T N and Tran D T (2011) Synthesis, antioxidant and antimicrobial activities of a novel series of chalcones, pyrazolic chalcones and allylic chalcones. *Pharm Pharmacy* **2**: 282-88.

Ezhilarasi R M, Jayachandramani N and Mahalakshmi S (2015) Microwave assisted synthesis and characterization of new 2-pyrazoline derivatives. *Inter J Med Chem Analysis* **5**(2): 93-98.

FRAC code list 1: Fungicidal sorted by FRAC code (2013) *imp.ifas.ufl.edu.*

Gothwal P and Srivastava Y K (2012) A facile microwave induced synthesis of some novel 3-[4'-(4''-nitrophenoxy)-phenyl]-5-(substituted aryl)-2-pyrazoline-1-carboxaldehydes as potential antimicrobial agents. *J Chem Biol Phys Sci* **2**: 622-27.

Grover R K and Moore J D (1962) Toxicometric studies of fungicides against brown rot organisms *Sclerotonia fructicola* and *Sclerotonia laxa*. *Phytopathology* **52**: 876-80.

Guantai E M, Ncokazi K and Egan T J (2011) Enone and chalcone-chloroquinoline hybrid analogues: in silico guided design, synthesis, antiplasmodial activity, *in vitro* metabolism, and mechanistic studies. *J Med Chem* **54**: 3637-649.

Hasan A, Rasheed L and Malik A (2007) Synthesis and characterization of variably halogenated chalcones and flavonols and their antifungal activity. *Asian J Chem* **19**(2): 937-48.

Huang Z Z and Wang L (2002) Novel synthesis of (E)-Fluorochalcones. *Synlett* **8**: 1281–282.

Jain U K, Bhatia R K, Rao A R, Singh R, Saxena A K and Sehar I (2014) Design and development of halogenated chalcone derivatives as potenrial anticancer agents. *Tropical J Phama Res* **13**(1): 73-80.

Jakhar and Makrandi (2013) Ecofriendly bromination of chalcone and synthesis of flavanole using grinding technique. *Ind J Chem* **52B**(1): 141-45.

Joshi V D, Kshirsagar M D and Singhal S (2012) Synthesis and antimicrobial activities of various pyrazolines from chalcones. *Ind J Chem Tech Res* **4**: 971-75.

Julio M A, Sylvia P, Diaz C, Josefina S, Francisco D V and Rivero I A (2009) Solution-phase parallel synthesis of substituted chalcones and their antiparasitary activity against *Giardia lamblia*. *Bioorg Med Chem* **17**: 6780–85.

Jyothi M V, Dinda S C, Reddy J R and Venkatesh P (2012) Synthesis and antimicrobial activity evaluation of some novel pyrazolines. *J Chem Pharm Res* **4**: 2626-30.

Kumar D, Kumar N M, Akamatsu K, Kusaka E, Harada H and Ito T (2010) Synthesis and biological evaluation of indolyl chalcones as antitumor agents. *Bioorg Med Chem* **20**: 3916-19.

Lawrence N J, Patterson R P, Ooi L L, Cook D and Ducki S (2006) Effects of α-substituents on structure and biological activity of anticancer chalcones. *Bioorg Med Chem* **16**: 5844-848.

Leroux F R (2016) *In Situ* generated fluorinated iminium salts as powerful tool in organic synthesis. *Proc 5th Inter Symposium on Organofluorine Compounds in Biomedical, Materials and Agricultural Sciences* Pp. 7, Jacobs University, Germany.

Makovitzki A, Viterbo A, Brotman Y, Chet I and Shai Y (2007) Inhibition of fungal and bacterial plant pathogens *in vitro* and in planta with ultrashort cationic lipopeptides. *Appl Environ Microbiol* **73**(20): 6629-36.

Mandge S, Singh H P, Gupta S D and Moorthy N S H N (2007) Synthesis and characterization of some chalcone derivatives. *Trends App Sci Res* **2(1)**: 52-6.

Nassar E (2010) Synthesis, (*in vitro*) antitumor and antimicrobial activity of some pyrazoline, pyridine, and pyrimidine derivatives linked to indole moiety. *J Am Sci* **6**:463-71.

Nene Y L and Thapiyal P N (1993) Fungicides in plant disease control. Pp. 525 Oxford and IBH Publishing Co Pvt Ltd New Dehli.

Nowakowska Z (2007) A review of anti-infective and anti-inflammatory chalcones. *Eur J Med Chem* **42**:125-37.

Panchal A D, Kunjadia P D and Patel P M (2011) Synthesis and biological evaluation of chalcone derivatives linked triazole. *Inter J Pharm Sci Drug Res* **3**(4): 331-37.

Patil C B, Mahajan S K and Katti S A (2009) Chalcone: A versatile molecule. *J Pharm Sci Res* **1**(3): 11-22.

Qian H and Dabin L (2011) Synthesis of chalcones *via* Clasien-Schmidt reaction catalyzed by sulfonic acid-functional ionic liquids. *Ind Eng Chem Res* **50**: 43-49.

Ramanarayan G V, Shukla V G and Akamanchi K G (2002) A novel and one step procedure of □-bromo-□□-unsaturated carbonyl compounds. *Synlett* **12**: 2059-61.

Rayar A, Veitia M S and Ferroud C (2015) An efficient and selective microwave-assisted Claisen-Schmidt reaction for the synthesis of functionalized benzalacetones. *Springer Plus* **4**: 1-5.

Rekhter M A, Grushetskaya G N, Panasenko A A and Krimer M Z (1995) New derivatives of N-vinyltriazoles: 1,3-diaryl-3-(1*H*-1,2,4-triazol-1-yl)-2-propen-1-ones. *Chem Heterocycl compound* **31**: 92-96.

Ritter M, Martins R M, Rosa S A, Malavolta J L, Lund R G, Floresd A F C and Pereira C M P (2015) Green synthesis of chalcones and microbiological evaluation. *J Braz Chem Soc* **26**(6): 1201-10.

Sebti S, Solhy A, Tahir R and Smahi A (2002) Modified hydroxyapatite with sodium nitrate, an efficient new solid catalyst for the Claisen-Schmidt condensation. *Appl Catalysis* **23**: 273-81.

Seelam N, Shrivastava S P and Prasanthi S (2013) Synthesis and antimicrobial activity of some novel fused heterocyclic moieties. *Org Commun* **6**(2): 78-85.

Shen-Jeu W, Cheng-Tsung L, Lo-Ti T, Jing-Ru W, Horng-Huey K, Jin-Pyang W and Chun-Nan L (2005) Synthetic chalcones as potential anti-inflammatory and cancer chemopreventive agents. *Eur J Med Chem* **40**(1): 103-12.

Singh P, Raj R and Kumar V (2012) 1,2,3- Triazole tethered beta-lactam-chalcone bifunctional hybrids: synthesis and anticancer evaluation. *Eur J Med Chem* **47**: 594-600.

Sinha S, Medhi B and Sehgal R (2013) Chalcones as an emerging lead molecule for antimalarial therapy: a review. *J Modern Med Chem* **1**:64-77.

Srivastava Y K (2008) Ecofriendly microwave assisted synthesis of some chalcones. *Rasayan J Chem* **1**(4): 884-86.

Susanne V, Susanne O, Gabi B and Jorg H (2008) Natural and non-natural prenylated chalcones: Synthesis, cytotoxicity and anti-oxidant activity. *Biorg Med Chem* **16**: 4286-293.

Tailor N K (2014) Synthesis and antifungal activity of certain chalcones and their reduction. *Indo Global J Pharma Sci* **4**(1): 2-5-8.

Tanvir H, Hamid L S, Muhammad Z R, Muhammad M Y and Masood P (2009) Anti-oxidant, anti-fungal and anti-leishmanial activities of novel 3-[4-(1*H*-imidazol-1-yl) phenyl] prop-2-en-1-ones. *Eur J Med Chem* **44**: 4654-60.

Tomar V, Bhattacharjee G, Kamaluddina and Kumar A (2007) Synthesis and antimicrobial evaluation of new chalcones containing piperazine or 2,5-dichlorothiophene moiety. *Bioorg Med Chem* **17**: 5321-24.

Varo J J, Patel D M, Parmar K C, Patel B M and Vasava S V (2014) Synthesis and antifungal activity of some novel chalcones containing pyrazole moiety. *Der Pharma Chemica* **6**(5): 166-170.

Vazquez-Rodriguez S, Matos M J and Santana L (2013) Chalcone-based derivatives as new scaffolds for hA3 adenosine receptor antagonists. *J Pharm Pharmacol* **65**(5):697-703.

Winter E, Pizzol C D and Locatelli C (2014) *In vitro* and *in vivo* effects of free and chalcones-loaded nanoemulsions: insights and challenges in targeted cancer chemotherapies. *Int J Environ Res Public Health* **11**(10):10016-35.

Yadav N, Dixit S K, Bhattacharya A, Mishra L C, Sharma M, Awasthi A K and Bhasin V K (2012) Antimalarial activity of newly synthesized chalcone derivatives. *Chem Biol Drug Des* **80**: 340-47.

Zhu S S, Liu X L, Liu P F, Li Y, Li Y, Li J Q, Wang H M, Yuan S K and Si N G (2007) Flumorph is a novel fungicide that distrupts microfilament organization in *Phytophthora melonis. Phytopathology* **97**(5): 643-49.

Zohreh N, Saeed E, Samaneh H, Sussan K A, Maryam N, Fatemeh P, Abbas S and Alireza F (2010) Novel antileishmanial chalconoids: Synthesis and biological activity of 1- or 3-(6-chloro-2*H*-chromen-3-yl)propen-1-ones. *Eur J Med Chem* **45**: 1424-29.

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