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Synthesis and *in vitro* biological activities of chalcones and their heterocyclic derivatives

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ABSTRACT

Chalcones have been reported to possess various biological activities such as anti-inflammatory, antioxidant, antitubercular, antibacterial activities. It is a basic moiety of many heterocyclic systems containing oxygen, sulphur and nitrogen. Nitrogen containing heterocyclic derivatives synthesized from chalcones have exhibited anti-inflammatory, antioxidant, antitubercular, antibacterial activities. Following Claisen-Schmidt condensation reaction, chloroacylaminochalcones ***(I & II)*** *were obtained by the reaction of chloroacetylamido acetophenones with substituted benzaldehyde. The chlorine atom of* ***(I*** *and* ***II)*** *was replaced by various amines to obtain substituted aminoacetamidochalcones* ***($I_{(a-d)}$*** *and* ***$II_{(a-d)}$)****. The Characterization of newly synthesized compounds done on the basis of UV, IR,* ^{1}*HNMR,* ^{13}*CNMR, Mass spectral data as well as elemental analysis. All the Synthesized compounds were evaluated for their anti-inflammatory, antioxidant, antitubercular and antibacterial activities.*

Keywords: Chalcones, Pyrimidine, Antitubercular activity, Anti-inflammatory activity, Antioxidant activity, Antibacterial activity.

INTRODUCTION

Chalcones are 1,3 diaryl-2-propene-1-one in which two aromatic rings are linked by α, β- unsaturated carbonyl system [1]. Chalcones derivatives are gaining much attention in the recent past because of their promising biological as well as pharmaceutical properties. In addition chalcones have been reported to exhibit several biological activities including antitumor, anti-inflammatory [2], immunomodulatory, antibacterial [3], antimalarial, antileishmanial, trypanocidal, antitubercular [4], antioxidant [5] and nitric oxide inhibitory activity. Nitrogen contains heterocyclic play an important role in medicinal chemistry. Pyrimidine derivatives are known to be biologically active compounds and substituted pyrimidines have shown wide range of biological activities like antitubercular [6-10], antioxidant activity [11], antibacterial [11-13] and anti-inflammatory [14].

RESULTS AND DISSCUSSION

Antitubercular activity

All the synthesized compounds were screened for *in-vitro* antitubercular activity by MABA method. All the derivatives exhibited activity at 100 µg/ml and 50 µg/ml respectively except **I, I_b, II_b** and **I_b** exhibited activity at 25 µg/ml. The results are given in the following table I.

Table I Antitubercular activity

Sl. No	Compounds	100µg/ml	50µg/ml	25µg/ ml	12.5µg/ Ml	6.25µg/ Ml	3.12µg/ml	1.6µg/ ml	0.8µg/ Ml
1	I	S	R	R	R	R	R	R	R
2	I_a	S	S	R	R	R	R	R	R
3	I_b	S	R	S	R	R	R	R	R
4	I_c	S	S	R	R	R	R	R	R
5	I_d	S	S	R	R	R	R	R	R
6	II	S	S	R	R	R	R	R	R
7	II_a	S	S	R	R	R	R	R	R
8	II_b	S	R	R	R	R	R	R	R
9	II_c	S	S	R	R	R	R	R	R
10	II_d	S	S	R	R	R	R	R	R

Antibacterial activity:

Antibacterial activity was tested for synthesized compounds by cup plate method. Moderate activity was observed for the tested organism at 100 µg/ml. Compound **I** and **I_a** exhibited good antibacterial activity towards the both gram negative and gram positive bacteria *Staphylococcus aureus* and *Klebsiella pneumonia* respectively. The results of antibacterial activity are depicted in table II.

Table II Antibacterial activity of all synthesized compounds

		Anti-bacterial activity (Zone of inhibition in mm)			
Sl.N.	**Compound code**	***S.aureus***	***B.subtilis***	***E.coli***	***K.pneumonia***
1	I	9	12	9	12
2.	I_a	12	10	10	11
3.	I_b	6	8	8	7
4.	I_c	-	12	8	9
5.	I_d	11	9	7	9
6.	II	10	8	10	8
7.	II_a	8	10	10	8
8.	II_b	7	10	7	7
9.	II_c	7	8	-	9
10.	II_d	-	7	6	7
Standard	Ampicillin	14	20	15	14

Anti-inflammatory activity:

Weak to moderate activity was seen at 10 µg/ml for most of the compounds. Compounds **II_b** with hydrazine and **II_d** with chloro as substituients exhibited good *in vitro* anti-inflammatory activity. The results are demonstrated in table III.

Table III Anti-inflammatory activity

S. N.	Compound code	% Inhibition	
		10µg/ml	50µg/ml
1	I	-	1.13
2	I_a	23.08	10.21
3	I_b	15.1 4	-
4	I_c	7.25	-
5	I_d	20.47	-
6	II	24.12	-
7	II_a	1.085	-
8	II_b	34.82	-
9	II_c	-	-
10	II_d	45.58	-
Standard	Indomethacin	94	96

Antioxidant activity: All the synthesized compounds tested using DPPH**,** the percentage inhibition of free radical are given in the following table IV

Table IV Antioxidant activity

Sl.No	Compound code	% Inhibition 10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml
1	I	2.98	15.54	40.14	44.27
2	I_a	3.98	8.09	9.58	11.38
3	I_b	34.71	35.03	37.91	40.71
4	I_c	-	-	-	-
5	I_d	-	4.25	6.49	-
6	II	-	-	-	-
7	II_a	-	-	-	-
8	II_b	23.64	54.20	76.03	87.11
9	II_c	-	-	-	-
10	II_d	-	-	-	-
Standard	Ascorbic acid	90	93	95	96

MATERIALS AND METHODS

All the melting points were determined in a Thermonik melting point apparatus and are uncorrected. The IR spectra of the synthesized compounds was recorded on a Fourier Transform IR spectrometer (model Shimadzu 8700) in the range of 400-4000 cm^{-1} using KBr pellets and ^1H NMR spectra was recorded on Amx - 400 MHz NMR spectrometer using $CDCl_3$ and the chemical shifts (δ) reported are in parts per million downfield using tetramethylsilane (TMS) as internal reference. ^{13}C-NMR spectra was recorded on Amx - 400 MHz NMR spectrometer using $CDCl_3$ and the chemical shifts (δ) reported are in parts per million downfield using tetramethylsilane (TMS) as an internal reference. Mass spectrum was recorded on Mass spectrophotometer (model Shimadzu) by LC-MS 2010A. The purity of the compounds was checked by thin-layer chromatography on silica gel G plates of 0.5 mm thickness as stationary phase and combination of n-hexane: ethyl acetate in different ratios as mobile phase. The UV spectra of the synthesized compounds were recorded on UV–Visible spectrophotometer (model Shimadzu 1601) using methanol and the values of absorption maxima (λ_{max}) were reported in nm. Elemental analysis were analysed by Thermo Finnigan Flash EA 1112 Series.

Preparation of *N*-(4-Acetylphenyl)-2-chloroacetamide:
4-amino acetophenone and glacial acetic acid were taken stirred well at room temperature. Solution of chloroacetyl chloride in glacial acetic acid was added to above reaction mixture drop wise with constant stirring. After addition was complete, stirring was continued for 30 minutes then added 0.4 M sodium acetate solution, the precipitate obtained was re-crystallized from ethanol.

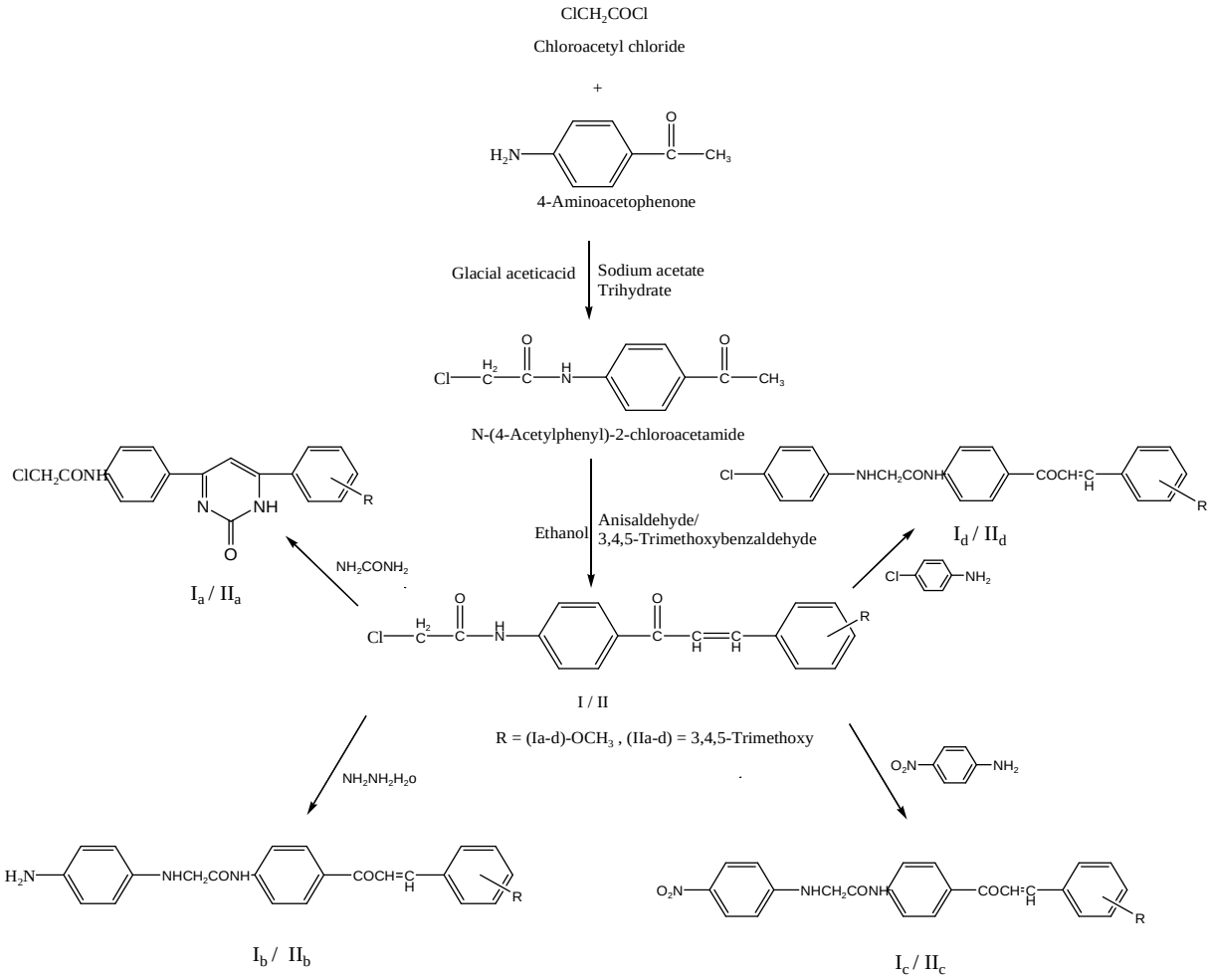
Preparation of 2-Chloro-*N*-{4-[3-(substitutedphenyl)-acryloyl]-phenyl}-acetamide (I, II): Equimolar quantity of *N*-(4-Acetylphenyl)-2-chloroacetamide and 4-methoxy benzaldehyde / 3,4,5-Trimethoxybenzaldehyde were dissolved in ethanol and 20 % NaOH solution was added slowly with stirring. After complete addition stirring was continued for 6 hours and left overnight. The reaction mixture was decomposed in ice-water, and adjusted to acidic pH . Crude product was recrystallized from ethanol.

Preparation of 2-Chloro-N-{4-[6-(substitutedphenyl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-phenyl}-acetamide (I_a, II_a):
Equimolar quantity of 2-Chloro-*N*-{4-[3-(substituted phenyl)-acryloyl]-phenyl}-acetamide and urea were dissolved in 30 ml of ethanol, refluxed for 6-8 hours with 20 % NaOH. Reaction mixture was cooled, the solid product **(I_a, II_a)** obtained was recrystalized from ethanol.

General method for preparation of *N*-{4-[3-(substitutedphenyl)-acryloyl]-phenyl}-2-(substituted phenylamino) acetamide (I_{b-d} and II_{a-d}):
Equimolar quantity of 2-Chloro-*N*-{4-[3-(substitutedphenyl)-acryloyl]-phenyl}-acetamide **(I / II)** and respective amines were dissolved in 30 ml of ethanol and refluxed for 3 hours. Reaction mixture was cooled to room temperature and poured into crushed ice to obtain product **(I_{b-d} and II_{a-d})** and re-crystallized from ethanol.

2-Chloro-*N*-{4-[3-(4-methoxyphenyl)-acryloyl]-phenyl}-acetamide (I):
Yellowish brown solid, m.p.124-26^oC; Yield: 63.28%; λ_{max}=203.20nm; IR (KBr) cm^{-1} 3437(NH str), 3014 (C=C), 2939 (CH_2), 1587 (C=O), 1506 (C=C), 1423 (C=C alkene), 1246 (C-O str), 596 (C-Cl).

Scheme of synthesis:



2-Hydrazino-*N*-{4-[3-(4-methoxy-phenyl)-acryloyl]-phenyl}-acetamide (I_b): Yellowish brown solid, m.p.116-18^{o}C; Yield: 70.33%; λ_{max}=209nm; IR (KBr) cm^{-1} 3431, 3371 (NH str), 1595 (C=O), 1448 (C=C alkene), 1300 (C-O str).

***N*-{4-[3-(4-Methoxy-phenyl)-acryloyl]-phenyl}-2-(4-nitro-phenylamino)-acetamide (I_c)**: Yellowish brown solid, m.p.136-38^{o}C; Yield: 46.97%; λ_{max}=209nm; IR (KBr) cm^{-1} 3470 (NH str), 2924 (CH_2), 1633 (C=O),1589 (CONH), 1469 (C=C alkene, C-NO_2), 1027 (C-O str). ^{1}HNMR (400 MHz, $CDCl_3$) δ 3.8 (s,3H,OCH_3), δ 4.3 (s, 2H) δ 7.57 (d, 1H, CH, J=8.76 Hz) δ 7.91 (d, 1H, CH J=8.6 Hz) δ 6.7-8.08 (13H, Ar H) δ 1.25 (S, 2H, CH_2).

2-(4-Chloro-phenylamino)-*N*-{4-[3-(4-methoxy-phenyl)-acryloyl]-phenyl}-acetamide (I_d): Yellow solid, m.p.140-42^{o}C; Yield: 65%; λ_{max}=203nm; IR (KBr) cm^{-1} 3446 (NH str), 2918 (CH_2), 1593 (C=O), 1508 (C=C Ar), 1429 (C=C alkene), 1294 (C-O str), 615 (C-Cl).

2-Chloro-*N*-{4-[3-(3,4,5 -trimethoxy-phenyl)-acryloyl]-phenyl}-acetamide (II): Yellowish brown solid, m.p.120-22^{o}C; Yield: 64.87%; λ_{max}=209.20nm; IR (KBr) cm^{-1} 3441 (NH str), 2931 (CH_2), 1639 (C=O), 1593 (CONH), 1421 (C=C Ar), 1323, (C-O str), 584 (C-Cl). ^{1}HNMR (400 MHz, $CDCl_3$) δ 7.5, 7.4 (d, 1H, 1H), δ 4.3 (s, 2H, CH_2), δ 8.0 (s, 1H, NH), δ 3.7 (m, 9H, OCH_3) δ 7.79-7.83 (m, 4H ArH), δ 6.36 (s, 2H, ArH). ^{13}CNMR (400 MHz $CDCl_3$) δ 123, 142 (C) δ 48.06 (CH_2) δ 56.52 (OCH3) δ 163, 187 (C=O) δ 146, 120, 120, 129, 323, 129, 129, 105, 148, 132, 148 (aryl carbons).

2-Chloro-N-{4-[2-oxo-6-(3,4,5-trimethoxy-phenyl)-1,2-dihydro-pyrimidin-4-yl]-phenyl}acetamide (II_a): Yellow solid, m.p.156-58^oC; Yield: 60.91%; λ_{max}=209.60nm; IR (KBr) cm^{-1} 3443 (NH str), 3061 (C-H Ar), 2929 (CH_2 alkane), 1589 (C=O), 1502 (C=N), 1276 (C-O str). 582 (C-Cl). ^{1}HNMR (400 MHz, $CDCl_3$) δ 4.27 (s, 2H, CH_2), δ 8.0 (s, 1H, NH), δ 3.7 (m, 9H, OCH_3) δ 7.6-7.7 (m, 4H ArH), δ 6.26 (s, 2H, ArH), δ 8.04 (m, 2H, NH, ArH). ^{13}CNMR (400 MHz $CDCl_3$) δ 126, 143 (C) δ 61.01 (CH_2) δ 56.52 (OCH3) δ 160, 188 (C=O) δ 105, 113, 35, 113, 93, 121, 40, 128, 40, 130, 86, 131, 10, 140, 151, 30, 152, 73, 153, 44 (aryl carbons):

2-Hydrazino-*N*-{4-[3-(3,4,5-trimethoxy-phenyl)-acryloyl]-phenyl}-acetamide (II_b): Brown solid, m.p.120-22^oC; Yield: 67.70%; λ_{max}=207.80nm.

2-(4-Nitro-phenylamino)-*N*-{4-[3-(3,4,5-trimethoxy-phenyl)-acryloyl]-phenyl}-acetamide (II_c): Yellow solid, m.p.118-20^oC; Yield: 38.52%; λ_{max}=207.80nm; IR (KBr) cm^{-1} 3470 (NH str), 2926($CH/CH_2/CH_3$), 1622 (C=O), 1589 (CONH), 1456 (C-NO_2, C=C), 1018,1116, 1170 (C-O str). ^{1}HNMR (400 MHz, $CDCl_3$) δ 7.4, 7.6 (d, 1H, 1H), δ 4.5 (s, 2H, CH_2), δ 4.2 (s, 1H, NH), δ 3.9 (m, 9H, OCH_3) δ 6.60-6.73 (m, 4H ArH), δ 6.86 (s, 2H, ArH), δ 7.92-7.95 (m, 2H, ArH), δ 8.04 (m, 2H, NH, ArH). ^{13}CNMR (400 MHz $CDCl_3$) δ 126, 143 (C) δ 61.01 (CH_2) δ 56.52 (OCH3) δ 160, 188 (C=O) δ 105, 113, 35, 113, 93, 121, 40, 128, 40, 130, 86, 131, 10, 140, 151, 30, 152, 73, 153, 44 (aryl carbons): LCMS: m/e = 491 (100 %). Calculated % = 63.67 (C), 5.10 (H), 8.57 (N). Found % = 63.63 (C), 5.34 (H), 8.07 (N).

2-(4-Chloro-phenylamino)-*N*-{4-[3-(3,4,5-trimethoxy-phenyl)-acryloyl]-phenyl}-acetamide (II_d): Yellow solid, m.p.160-62^oC; Yield: 52.17%; λ_{max}=210nm; IR (KBr) cm^{-1} 3464 (NH str), 3221 (C-H Ar), 3340 (NH), 2933 (CH_2), 1583 (C=O), 1454 (C=C alkene), 1280 (C-O str), 586(C-Cl). ^{1}HNMR (400 MHz, $CDCl_3$) δ 7.5, 7.9 (d, 1H, 1H), δ 4.0 (s, 2H, CH_2), δ 4.0 (s, 1H, NH), δ 3.7 (m, 9H, OCH_3) δ 6.18-6.24 (m, 4H ArH), δ 6.26 (s, 2H, ArH), δ 7.92-7.95 (m, 2H, ArH), δ 8.04 (m, 2H, NH, ArH). ^{13}CNMR (400 MHz $CDCl_3$) δ 126, 148 (C) δ 61.01 (CH_2) δ 56.52 (OCH3) δ 160, 187 (C=O) δ 105, 113, 35, 113, 93, 121, 40, 128, 40, 130, 86, 131, 10, 140, 151, 30, 152, 73, 153, 44 (aryl carbons).

ANTITUBERCULAR ACTIVITY

The antitubercular activity of compounds was assessed against *M. Tuberculosis* using Microplate Alamar Blue Assay (MABA). Briefly, 200 µl of sterile 96 wells plate was taken to minimize evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg /ml. Plates were covered and sealed with parafilm and incubated at 37^oC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10 % tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.

ANTIBACTERIAL ACTIVITY

Microbial study was conducted by cup plate method. The drugs were initially dissolved in DMSO and tested at concentrations of 100 µg /ml against all the microorganisms.

Sterile NA/SDA plates were prepared and 0.1 ml of the inoculum from standardized culture of test organism was spread uniformly. Wells were prepared by using a sterile borer of diameter 10 mm and 100 µl of the test substance, standard antibiotic and the solvent control were added in each well separately. Standard antibiotic, Ampicillin were tested against gram -ve, gram +ve bacteria respectively. The plates were placed at 4 ºC for 1 h to allow the diffusion of test solution into the medium and plates were incubated at a temperature optimal for the test organism and for a period of time sufficient for the growth of at least 10 to 15 generations (usually 24 h at 37 ºC). The zone of inhibitions of microbial growth around the well was measured in mm.

ANTI-INFLAMMATORY ACTIVITY

A solution of 0.2 % w/v of Bovine serum albumin (BSA) was prepared in Tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid. Stock solutions of 1000 µg /ml of all test samples were prepared by using methanol as a solvent. From the stock solutions two different concentrations of 10 µg /ml and 50 µg /ml were prepared by using methanol as a solvent. 10 µg /ml of each test sample was taken to which 5ml of 0.2 % BSA was added. The control consists of 5ml 0.2 % w/v BSA solution with 0.1ml methanol. The 0.1ml standard consisted 100 µg /ml of Indomethacin in methanol with 5ml 0.2 % w/v BSA solution. The volumetric flasks were heated at 72°C for 5 minutes and then cooled for 10 minutes. The absorbance of these solutions was determined by using

spectrophotometer at a wavelength of 660 nm. The % denaturation of the protein (% inhibition) was determined by using formula as given.

$$\% \text{ inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

Antioxidant activity:

2, 2-diphenyl-1-picryl hydrazine (DPPH) method was adopted, a stock solution of 1 mg/ml of ascorbic acid dissolved in methanol was prepared, dilutions of different concentrations 10 to 40 µg / ml were obtained. One ml each of these dilutions was taken in different volumetric flasks to which 1 ml of DPPH solution was added and volume was made up to 10 ml. The test solution were prepared in similar manner as that of standard ascorbic acid and the absorbance were recorded at 516 nm after duration of 30 min. The percentage of inhibition of free radical was calculated following formula as given below.

$$\% \text{ inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

CONCLUSION

From research study the desired compounds were obtained from method adopted and established by spectral data. All the chalcones and heterocyclic derivatives have shown good to moderate antitubercular, antibacterial, anti-inflammatory and antioxidant activities. Trimethoxy (electron releasing) phenyl substituents of chalcones as shown better results towards inflammation , while chalcones and heterocyclic derivatives (pyrimidine moiety) irrespective of any substitution have shown antitubercular activity. Chalcones and heterocyclic derivatives exhibited antibacterial activity towards the strain used (except for E.coli).

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