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Synthesis, characterization and pharmacological evaluation of chalcones and its derivatives for analgesic activity

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***Abstract*---**Chalcones have a simple privileged scaffold and are active lead molecules in medicinal chemistry for the discovery of new drugs. Chalcone is the organic compound $C_6H_5C(O)CH=CHC_6H_5$. It is an α, β-unsaturated ketone. Chalcones and their derivatives have a huge importance in medicinal chemistry, displaying a wide range of pharmacological activities including analgesic activites. A variety of important biological compounds are known collectively as chalcones or chalconoids. In this view medicinal chemists are also trying to speed up drug discovery process for finding the lead molecule (Thomas *et al.* 1998). The compounds with chalcone as backbone have been reported to possess varied biological and pharmacological activities, including antimicrobial, anti-inflammatory, analgesic, cytotoxic, antitumor, antimalarial, antitubercular, antiviral, anti-HIV, antiulcerative, antileishmanial activities.All the newly synthesized pyrimidines were characterized by means of IR, ^{1}H- and ^{13}C-NMR, Electron Ionization (EI)-mass and elemental analyses and screened for analgesic activities. Pyrimidines also displayed better analgesic activity.

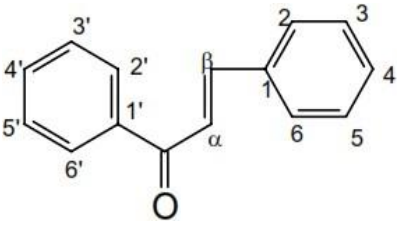
***Keywords*---**Chalcone, Pyrazolines,1,3,5-trisubstituted pyrazolines, Claisen–Schmidt condensation, Pharmacological evaluation, Analgesic.

Introduction

Medicinal chemistry is the application of chemical research techniques to the synthesis of pharmaceuticals. During the early stages of medicinal chemistry development, scientists were primarily concerned with the isolation of medicinal agents found in plants. Chalcones are also known as benzyl acetophenone or

benzylidene acetophenone. In chalcones, two aromatic rings are linked by an aliphatic three carbon chain. Chalcones (trans-1, 3-diaryl-2-propen-1-ones) are α, β-unsaturated ketones consisting of two aromatic rings (ring A and B) having diverse array of substituents. Rings are interconnected by a highly electrophonic three carbon α, β-unsaturated carbonyl system that assumes linear or nearly planar structure Chalcone is synthesized by Claisen-Schmidt condensation, which involves the cross-aldol condensation of aldehydes and ketones with a base or acid catalyst followed by a dehydration reaction.

General structure of chalcone:



Chalcones are one of the major classes of natural products which occur widely in nature particularly in colored flowers and wide spread distribution in fruits, vegetables, spices and tea. Various natural or synthetic chalcones have been found to posses diverse biological activites (Di Carlo *et al.* 1999).
All the chalcones give dark red coloration with concentrated sulphuric acid wilson test and violet red coloration with alcoholic ferric chloride solution. Chalcones on heating with traces of iodine in dimethylsulphoxide (DMSO) for two hours give the corresponding flavones. Pain has been defined as a particular type of sensory experience perceived by nerve tissue distinct from sensation such as touch, heat and cold. It acts as'a signal against disturbances either in the body or in the external environment of an individual. Drugs which alter the pain sensitivity or relieves pain are of 2 types:
a) Non-narcotic (e.g. Salicylates)
b) Narcotic (e.g. Opium alkaloids)

In general three types of stimuli - physical, thermal and chemical are employed in mice or rats for evaluation of analgesic property of the compound. (Lavie, 1999; Eddy and Leimtach, 1953).

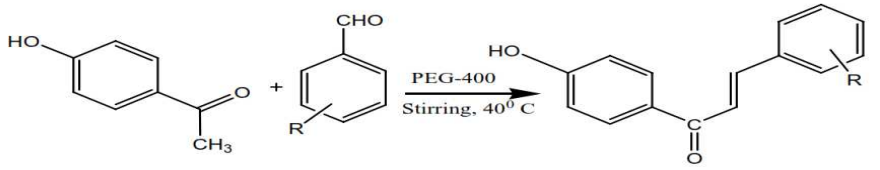
Material and Methods

The following methods for synthesizing chalcones and Chalcones derivatives. All the synthetic compounds were acquired from Sigma-Aldrich, Spectrochem and High Media.. Melting point is determined by utilizing an open capillary and are uncorrected.TLC were performed on silica plates with observation under uv or iodine chamber . Infrared Infrared spectra were recorded on a FT-IR Shimadzu DZU 8400S spectrophotometer in KBr circles and Elemental examination were done on a Perkin-Elmer 2400C, H, N analyzer and values were viewed to be within satisfactory limits reaches of the determined qualities. The 1H-NMR spectra of the methodize mixtures in CDCl3/DMSO were recorded at 400 MHz

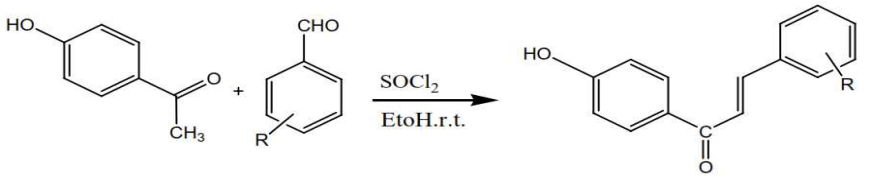
by Bruker Advance II 400 NMR spectrometer. Chemical shift esteems are given in scale utilizing tetramethylsilane (TMS) as an inside norm. Huge 1H-NMR information are written all together: number of protons, assortment (b, wide; s, singlet; d, doublet; t, trio; m, multiplet), coupling constants in Hertz, task. The fab mass spectra (at room temperature) were recorded on tof MS-ES Mass spectrometer.

General methods of synthesis of chalcones

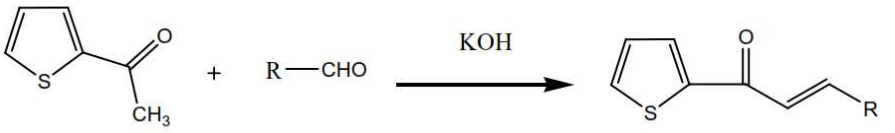
Chalcones are well known intermeadiates for synthesizing various heterocyclic compounds. They can be obtained by the acid or base catalyzed aldol condensation of acetophenones with benzaldehydes (Guida *et al.* 1997).
1) Claisen–Schmidt condensation between 4-hydroxy acetophenone and benzaldehyde was carried out in the presence of a base catalyst was stirred in PEG-400 as a recyclable solvent form 4' -hydroxy chalcones (Sreedhar *et al.* 2010).



2) Stirred mixture of 4-hydroxy acetophenone and various benzaldehyde in the presence of thionylchloride in absolute ethanol form substituted 4'-hydroxy chalcones (Eddarir, 2003).

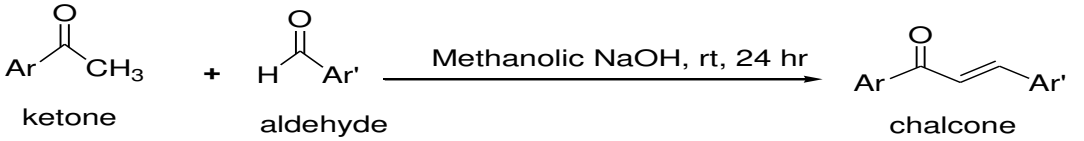


A mixture of 2-acetyl thiophene substituted aldehydes was stirred in ethanol then an aqueous solution of KOH was added to form chalcones (Romanelli *et al.* 2011).



1. General method of synthesis of chalcone derivatives (1a-1p)

Chalcones are synthesized by Claisen-Schmidt condensation (Furniss *et al.,* 1989; Kumar *et al.*, 2010) of aldehyde and ketone by base catalyzed or acid catalyzed followed by dehydration to yield chalcones (Figure 1).



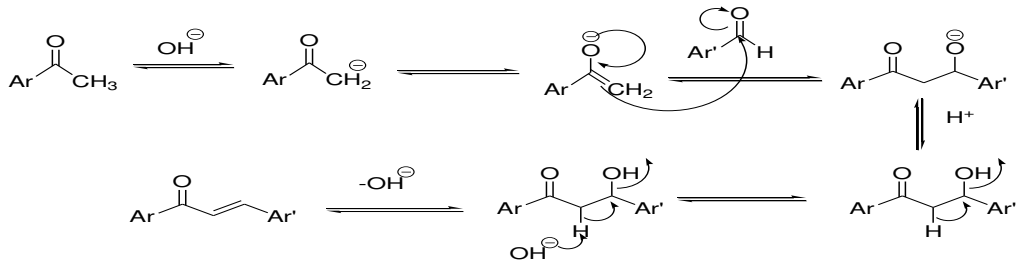


Figure 1. : Mechanism of reaction for synthesis of chalcone derivatives (1a-1p)

The synthesis of the designed compounds (**2a-2p, 3a-3p**) was performed in a manner as outlined in Figure 1.1-1.2 and Table 1.

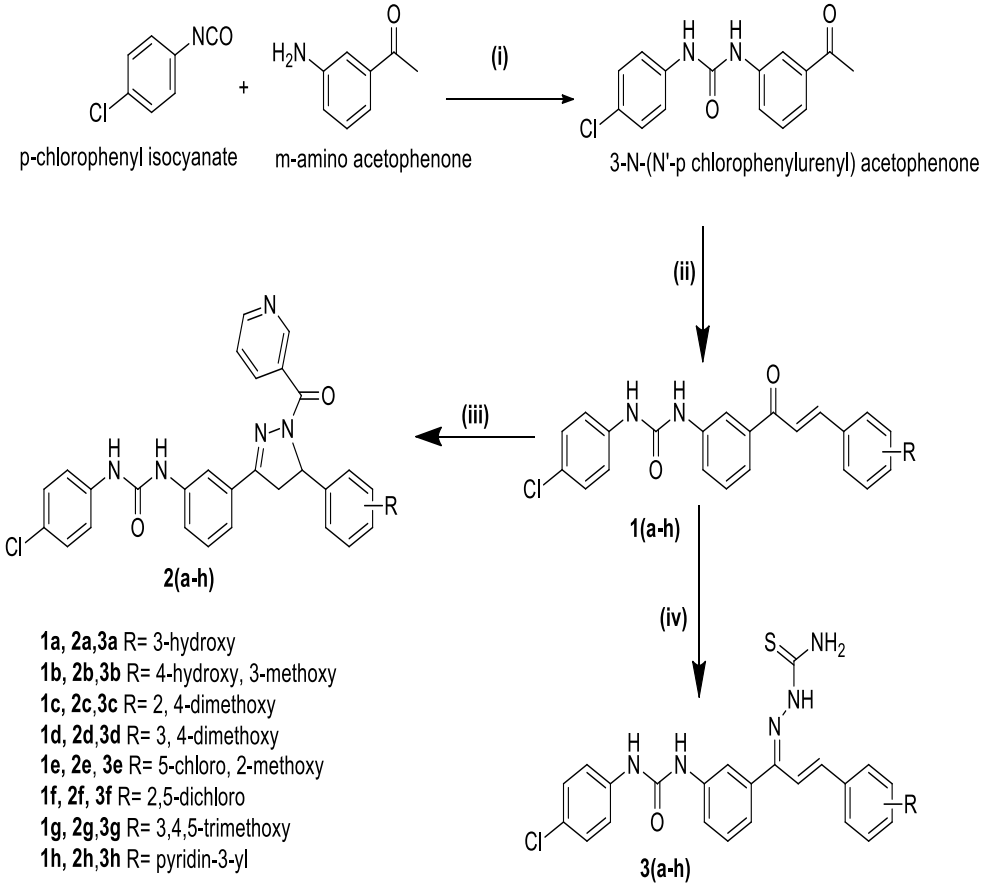


Figure. 1.1: The synthesis of the designed compounds 1a-1h, 2a-2h, 3a-3h (i) Me_2CO, rt, 6 hr (ii) substituted benzaldehyde, methanolic NaOH, stirred at room temperature, 24 hr (iii) *n*-butanol, reflux (iv) thiosemicarbazide, EtOH, ACOH, reflux.

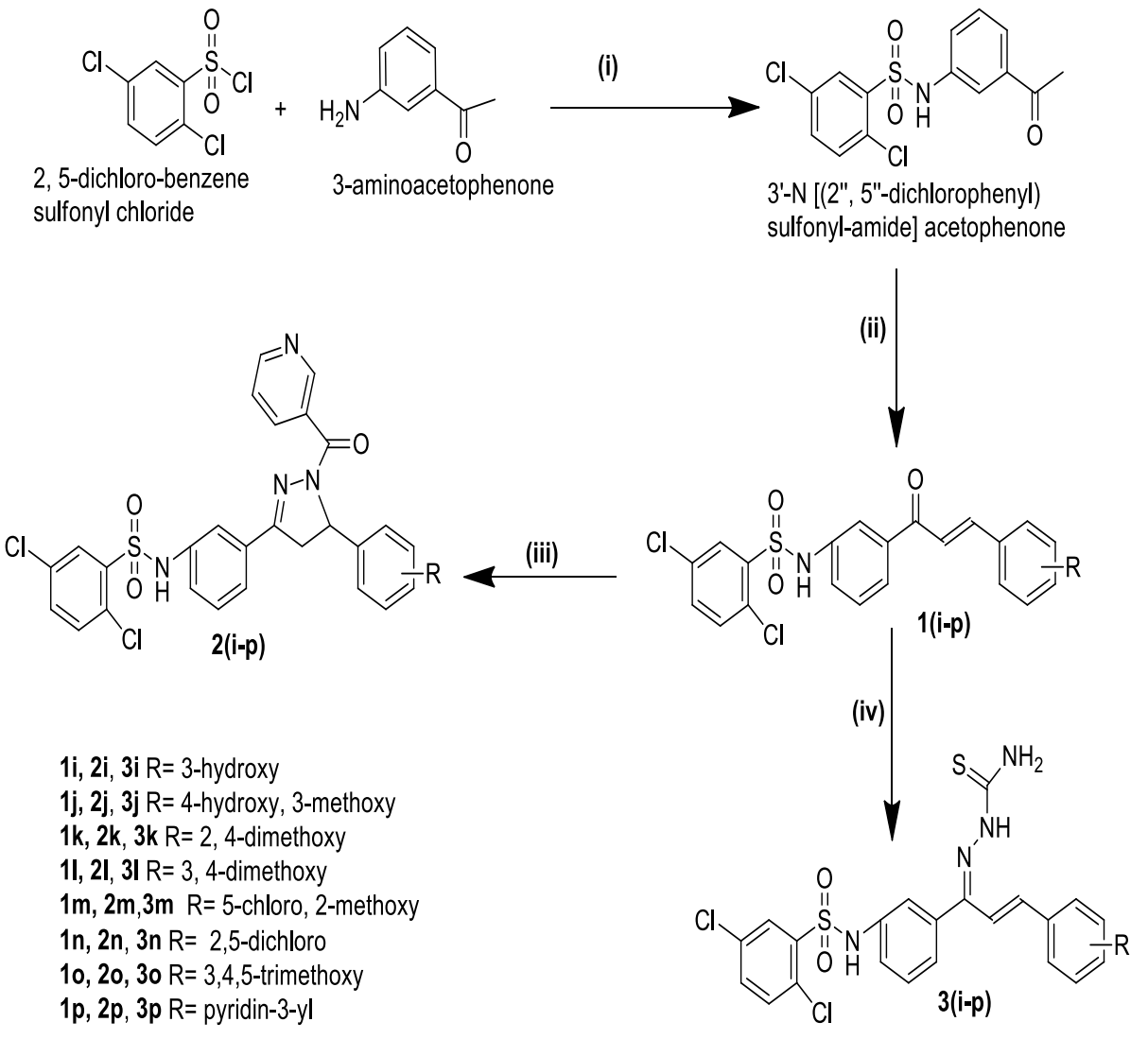
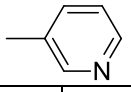
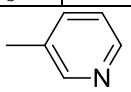


Figure. 1.2: The synthesis of the designed compounds 1i-1p, 2i-2p, 3i-3p (i) $CHCl_3$, rt, 3-6 hrs (ii) substituted benzaldehyde, methanolic NaOH, stirred at room temperature, 24 hr (iii) *n*-butanol, reflux (iv) thiosemicarbazide, EtOH, ACOH, reflux.

Table.1: Different substitutions on new synthesized substituted Chalcones and pyrazolines compounds (1a- 1p, 2a-2p, 3a-3p)

S.No	Comp. No.		**R_2**	**R_3**	**R_4**	**R_5**
1	2a	3a	-	OCH_3	-	-
2	2b	3b	-	OCH_3	OH	-
3	2c	3c	OCH_3	-	OCH_3	-
4	2d	3d	-	OCH_3	OCH_3	-
5	2e	3e	OCH_3	-	-	Cl

6	2f	3f	Cl	-	-	Cl
7	2g	3g	-	OCH_3	OCH_3	OCH_3
8	2h	3h				
9	2i	3i	-	OCH_3	-	-
10	2j	3j	-	OCH_3	OH	-
11	2k	3k	OCH_3	-	OCH_3	-
12	2l	3l	-	OCH_3	OCH_3	-
13	2m	3m	OCH_3	-	-	Cl
14	2n	3n	Cl	-	-	Cl
15	2o	3o	-	OCH_3	OCH_3	OCH_3
16	2p	3p				

2. Synthesis of intermediates

Synthesis 3-N-(N'-p-chlorophenylurenyl)acetophenone

Synthesis of methyl ketone derivative was carried out by making *m*-amino acetophenone react with the *p*-chlorophenyl isocyanate. A mixture of the *m*-aminoacetophenone (2.7 g, 20 mmol) and p-chlorophenyl isocyanate (3 g, 20 mmol) was dissolved in dry acetone (100 mL). The mixture was stirred for 6-7 hr at room temperature, filtered, and the crude compound urenylacetophenone was recrystallized using ethanol (Sonmez *et al.,* 2011).

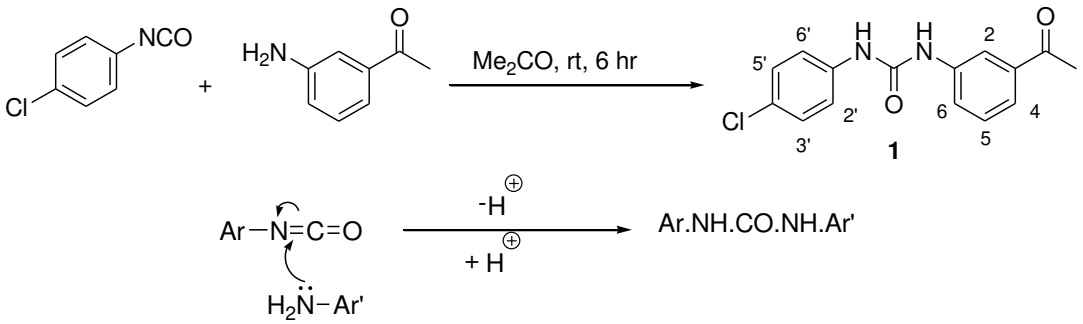


Figure 2 : Scheme for synthesis of 3-N-(N'-p-chlorophenylurenyl)acetophenone

Yield 3.3 g, 58%, White solid; mp 272-274 ^{o}C; IR(KBr) ν_{max} /cm^{-1} 3372 (N-H), 3056 (ArC-H), 2962 2872 (C-H), 1711 ($COCH_3$), 1645 (C=O), 1614, 1534, 1461 (Ar C=C), 1515, 1290, 1185 (ArC-N), 1147 (Ar-Cl) 756, 687 (Ar); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ_H 9.12 (br s, 1H, NH), 8.91 (br s, 1H, NH); 8.18 (1H, s, H-2), 7.78 (1H, d, *J* 5.9, H-6), 7.53 (3H, m, H-4, 2', 6'), 7.30 (1H, t, *J* 6.30, H-5), 7.21 (2H, d, *J* 6.65, H-3', 5'), 2.53 (s, 3H, 3-$COCH_3$).

Synthesis of 3'-N [(2'', 5''-dichlorophenyl) sulfonyl-amide] acetophenone

The intermediate compound 3'-N[(2'',5''-dichlorophenyl) sulfonyl-amide] acetophenone was synthesized adopting the procedure described by Leon *et al.* (2007) with some modifications (Figure 4.4).

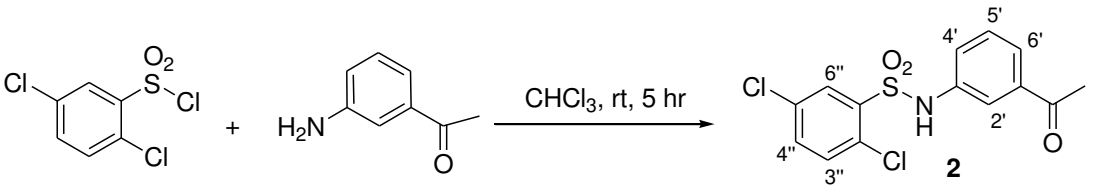


Figure 3: Scheme for synthesis of 3'-N[(2",5"-dichlorophenyl) sulfonyl-amide] acetophenone

A mixture of 3-aminoacetophenone (2.7 g, 20 mmol) and 2, 5-dichloro-benzene sulfonyl chloride (4.9 g, 20 mmol) in 5 mL of chloroform was stirred at room temperature (rt) for 3–6 hr. The resulting precipitate was washed with acetone, filtered, and the crude material obtained was recrystallized in acetonitrile to give pure compound 3'-N[(2",5"-dichlorophenyl) sulfonyl-amide] acetophenone.

Yield 3.6 g, 52%, Brown crystals; mp 230–232 °C; IR 3216 (N-H); 1667 (C=O); 1715 ($COCH_3$), 1337, 1270 (SO_2), 1142 (Ar-Cl), 3060 (Ar-H), 2967 (C-H), 1584, 1461, 1357, 1297, 1273, 1166, 993, 852, 819, 795, 720 (Ar); ^{1}H-NMR: δ_H 11.38 (s, 1H, NH), 7.94 (1H, s, H-6´´), 7.70 (1H, d, *J* 8.44, H-3´´), 7.25-7.44 (3H, m, H-2´, 5´, 6'), 7.71 (d, 1H, *J* 6.42, H-4´´), 6.94 (1H, d, *J* 8.91, H4'), 2.51 (s, 3H, CH_3CO).

General procedure for the synthesis of chalcone derivatives (1a-1p)

To a solution of substituted acetophenone (16 mmol) in 10 mL of methanol on an ice bath, freshly prepared 2 N methanolic NaOH solution (60 mL) was added and stirred for 10 min. To this, appropriate aldehyde (16 mmol) was added and stirred at room temperature for 12-24 hr. The reaction mixture was cooled on an ice bath, neutralized with diluted HCl and the precipitate was washed three times with 50 mL distilled water to give the crude product. The product was recrystallized from methanol or ethanol/ water.
The purity of the product was checked by TLC using ethyl acetate and hexane (4:6) as mobile phase and iodine vapors as detecting agent.
(E)-1-(4"-chlorophenyl)-3-(3-(3'-(3-hydroxyphenyl)acryloyl)phenyl)urea (1a)
(E)-1-(4"-chlorophenyl)-3-(3-(3'-(4-hydroxy-3-methoxyphenyl)acryloyl)phenyl)urea (1b)
Synthesis of (E)-1-(4"-chlorophenyl)-3-(3-(3'-(2,4-dimethoxyphenyl)acryloyl) phenyl)urea (1c)
Synthesis of (E)-1-(4"-chlorophenyl)-3-(3-(3'-(3,4-dimethoxyphenyl)acryloyl) phenyl)urea (1d)
Synthesis of (E)-1-(3-(3-(5-chloro-2-methoxyphenyl)acryloyl)phenyl)-3-(4-chloro-phenyl)urea (1e)
Synthesis of (E)-1-(4"-chlorophenyl)-3-(3'-(3-(2,5-dichlorophenyl)acryloyl) phenyl)urea (1f)
Synthesis of (E)-1-(4"-chlorophenyl)-3-(3'-(3-(3,4,5-trimethoxyphenyl) acryloyl) phenyl)urea (1g)
Synthesis of (E)-1-(4"-chlorophenyl)-3-(3'-(3-(pyridin-3-yl)acryloyl) phenyl) urea (1h)
Synthesis of (E)-2",5"-dichloro-N-(3'-(3-(3-methoxyphenyl)acryloyl)phenyl)benzene sulfonamide (1i)
Synthesis of (E)-2",5"-dichloro-N-(3'-(3-(3-hydroxy,4-methoxyphenyl)acryloyl)phenyl) benzenesulfonamide (1j)

Synthesis of (E)-2",5"-dichloro-N-(3'-(3-(2,4-dimethoxyphenyl)acryloyl)phenyl) benzene sulfonamide (1k)
Synthesis of (E)-2",5"-dichloro-N-(3'-(3-(3,4-dimethoxyphenyl)acryloyl) phenyl) benzene sulfonamide (1l)
Synthesis of (E)-2",5"-dichloro-N-(3'-(3-(5-chloro-2-methoxyphenyl)acryloyl)phenyl) benzenesulfonamide (1m)
Synthesis of (E)-2",5"-dichloro-N-(3'-(3-(2,5-dichlorophenyl)acryloyl)phenyl) benzenesulfonamide(1n)
Synthesis of (E)-2",5"-dichloro-N-(3'-(3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)benzene sulfonamide (1o)
Synthesis of (E)-2",5"-dichloro-N-(3'-(3-(pyridin-3-yl)acryloyl)phenyl) benzene sulfonamide (1p)**]**

General method for synthesis of 1, 3, 5-trisubstituted pyrazolines (2a-2p)

1,3,5-trisubstituted pyrazolines (**2a-2p**) were synthesized according to the scheme depicted in Figure 4.6 (Ozdemir *et al.,* 2008). In this method, chalcone and nicotinic acid hydrazide were refluxed in *n*-butanol in order to synthesize the desired product (Kini and Gandhi, 2008). Factors such as the structure and position of the substituents have profoundly influenced the rate of the reaction. The generally accepted interpretation of this reaction, involves the initial formation of an aryl hydrazone with subsequent nucleophilic attack of nitrogen upon the carbon-carbon double bond at position. Hence the electropositive nature of carbon may control the overall rate of the reaction. The electropositive nature of carbon is controlled by the aromatic ring directly connected to it. Halogens being electron withdrawing in nature significantly increase the positive character of carbon lead to faster reaction while electron donating alkyl and alkoxy groups contributed for slower reaction.

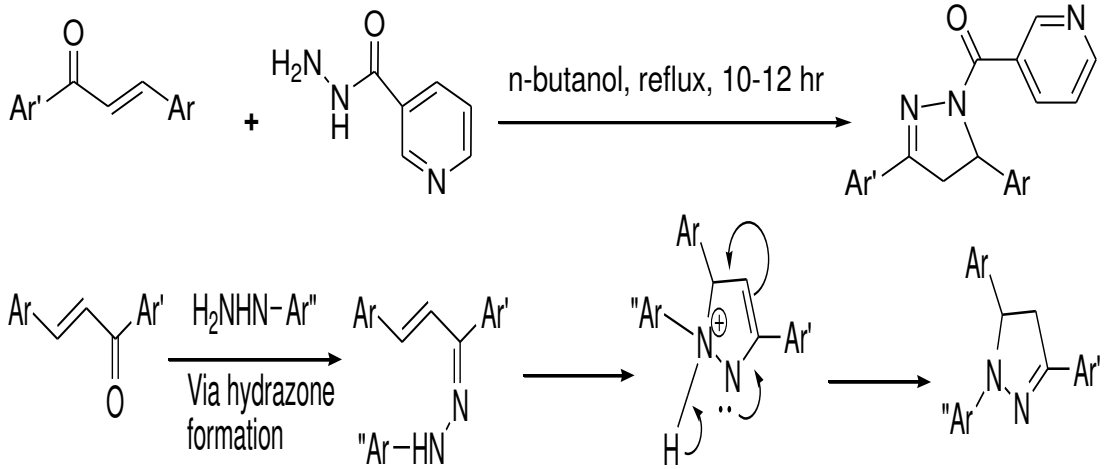


Figure 4. Scheme and mechanism of reaction for synthesis of compounds (2a-2p)

To the solution of the appropriate chalcone **1a-1p** (4 mmole) in 10 mL of *n*-butanol, (0.55 g, 4 mmole) of nicotinic acid hydrazide was added and the reaction mixture was refluxed for 8–10 hr. The excess of solvent was removed under reduced pressure and the reaction mixture was cooled on an ice bath. The products precipitated out at low temperature were washed five times with 50 mL

distilled water, reconstituted in minimum amount of methanol and dried under reduced pressure. This product was further purified by crystallization from the ethanol-DMF mixture (1:1). Purity of the products was checked by TLC using mixture of acetone and petroleum ether (40:60 V/V) as mobile phase.

[a-(4”-chlorophenyl)-c-(3-(5"-(3'-hydroxyphenyl)-1-nicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)urea (2a)
a-(4”-chlorophenyl)-c-(3-(5"-(4’-hydroxy,3'-methoxyphenyl)-1-nicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)urea (2b)
a-(4”-chlorophenyl)-c-(3-(5-(2',4'-dimethoxyphenyl)-1-nicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)urea (2c)
a-(4”-chlorophenyl)-c-(3-(5-(2',4'-dimethoxyphenyl)-1-nicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)urea (2d)
a-(4”-chlorophenyl)-c-(3-(5-(3',4'-dimethoxyphenyl)-1-nicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)urea (2e)
a-(4”-chlorophenyl)-c-(3-(5-(2',5'-dichloroyphenyl)-1-nicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)urea (2f)
a-(4”-chlorophenyl)-c-(3-(5-(3',4’,5’-trimethoxyphenyl)-1-nicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)urea (2g)
a-(4”-chlorophenyl)-c-(3-(5-(pyridine-3’-yl)-1-nicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)urea (2h)
2”,5”-dichloro-N-(3-(5-(3'-hydroxyphenyl)-1-nicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)benzenesulphonamide (2i)
2”,5”-dichloro-N-(3-(5-(4’-hydroxy,3'-methoxyphenyl)-1-nicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)benzenesulphonamide (2j)
2”,5”-dichloro-N-(3-(5-(2',4'-dimethoxyphenyl)-1-nicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)benzenesulphonamide (2k)
2”,5”-dichloro-N-(3-(5-(3',4'-dimethoxyphenyl)-1-nicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)benzenesulphonamide (2l)

General procedure for synthesis of thiosemicarbazide derivatives (3a-p)

A mixture of chalcones (3a–3p) (0.5 mmol) and thiosemicarbazide (0.5 mmol) in hot ethanol (50 mL) had a few drops of concentrated hydrochloric acid added. The reaction mixture was stirred atreflux temperature for 2–6 hr, and monitored by TLC using hexane : ethyl acetate (8:2) as the eluent. Afterwards, the precipitate was filtered off and the crude product purified by recrystallization from ethanol, resulting in the target compounds (3a–3p).

[(Z)-2-((E)-1-(3-(3-(4-chlorophenyl)ureido)phenyl)-3-(3hydroxyphenyl)allylidene) hydrazine carbothioamide (3a)
(Z)-2-((E)-1-(3-(3-(4-chlorophenyl)ureido)phenyl)-3-(4-hydroxy-3-methoxyphenyl) allylidene)hydrazine carbothioamide (3b)
(Z)-2-((E)-1-(3-(3-(4-chlorophenyl)ureido)phenyl)-3-(2,4-dimethoxyphenyl)allylidene) hydrazinecarbothioamide (3c)
(Z)-2-((E)-1-(3-(3-(4-chlorophenyl)ureido)phenyl)-3-(3,4-dimethoxyphenyl)allylidene) hydrazinecarbothioamide (3d)
(Z)-2-((E)-3-(5-chloro-2-methoxyphenyl)-1-(3-(3-(4-chlorophenyl)ureido)phenyl) allylidene)hydrazinecarbothioamide (3e)
(Z)-2-((E)-1-(3-(3-(4-chlorophenyl)ureido)phenyl)-3-(2,5-

dichlorophenyl)allylidene) hydrazinecarbothioamide (3f)
(Z)-2-((E)-1-(3-(3-(4-chlorophenyl)ureido)phenyl)-3-(3,4,5-trimethoxyphenyl) allylidene)hydrazinecarbothioamide (3g)
(Z)-2-((E)-1-(3-(3-(4-chlorophenyl)ureido)phenyl)-3-(pyridin-3-yl)allylidene)hydrazine carbothioamide (3h)
(Z)-2-((E)-1-(3-(2,5-dichlorophenylsulfonamido)phenyl)-3-(3-hydroxyphenyl) allylidene)hydrazinecarbothioamide (3i)
(Z)-2-((E)-1-(3-(2,5-dichlorophenylsulfonamido)phenyl)-3-(4-hydroxy-3methoxy phenyl) allylidene)hydrazinecarbothioamide (3j)
(Z)-2-((E)-1-(3-(2,5-dichlorophenylsulfonamido)phenyl)-3-(2,4-dimethoxyphenyl) allylidene)hydrazinecarbothioamide (3k)
(Z)-2-((E)-1-(3-(2,5-dichlorophenylsulfonamido)phenyl)-3-(2,4-dimethoxyphenyl) allylidene)hydrazinecarbothioamide (3l)
(Z)-2-((E)-3-(5-chloro-2-methoxyphenyl)-1-(3-(2,5-dichlorophenylsulfonamido) phenyl)allylidene)hydrazinecarbothioamide (3m)
(Z)-2-((E)-3-(2,5-dichlorophenyl)-1-(3-(2,5-dichlorophenylsulfonamido)phenyl) allylidene) Hydrazine carbothioamide (3n)
(Z)-2-((E)-1-(3-(2,5-dichlorophenylsulfonamido)phenyl)-3-(3,4,5-trimethoxyphenyl) allylidene)hydrazinecarbothioamide (3o)
(Z)-2-((E)-1-(3-(2,5-dichlorophenylsulfonamido)phenyl)-3-(pyridin-3-yl)allylidene) hydrazine carbothioamide (3p)**]**

Pharmacological Evalution

Physical methods

***Tail clip method*:** In this method an artery clip with thin rubber sleeves is applied to the base of the mouse tail for 30 seconds. Control mice make continuous efforts to dislodge the clip by biting it. Analgesics make the mice indifferent to the clip.
A group of mice is injected subcutaneously with a dose of the test substance and 30 minutes later the clip is applied. The percentage of mice in a group that fails to respond is calculated.

Thermal methods

***Hot plate method*:** Mice or rats are placed on a hot plate maintained at 55°C. The reaction time is that between placing the animals on the hot plate and licking of the fore or hind paws. The mean increase in the reaction time is plotted against time after drug administration and the area under the time response curve is calculated either graphically or planimetrically.
***Tail flick response*:** Mice or rats are held in suitable restrainer with tail protruding
out. Radiant heat is applied over the tail on a single spot with the help of suitable device. The time taken by the animal to withdraw (flick) the tail is taken as the reaction time, and the time response curve may be plotted.
Tail immersion test: Mice or rats are held in position in a suitable restrainer with tail protruding out. The tail upto 5 cm is dipped in a beaker of water at 55°C

and the time taken to withdraw the tail clearly out of water is taken as the reaction time.

Electric methods

Electrodes are implanted or introduced into the skin, scrotum, tail or ear of the experimental animals. The voltage that has to be applied across the electrode (terminals) in order to cause the animals to squeak or struggle is recorded before and after administration of the substance under test.

Chemical methods

It involves injection of phenylquinone, benzoquinone, acetic acid or bradykinin in mice through intraperitoneal route and the response to the irritant solution is a characteristic writhing, it is estimated by counting the number of total writhes in a group of 6 mice or rats.

Experimental / Methodology:

Tail immersion test method / tail flick method was adopted for evaluation of analgesic activity of the test compounds, the tail of the control, standard and test group animals (rats) was dipped in a beaker of water maintained 55+1°C and the time taken to withdraw the tail clearly out of water is taken as the reaction time.

Requirements:

a) Animals: Albino rats of either sex weighing between 150-200 gm, 72 numbers.
b) Standard drug: Ibuprofen suspension in 2% v/v tween 80 solution administered orally at the dose of 100 mg/kg body weight.
c) Samples: Test compounds were suspended in 2% v/v tween 80 solution and administered orally at the dose of 100 mg/kg body weight.
d) Water was heated in a beaker and the temperature was maintained at 55+1 °C.

Working procedure:

1. 72 albino rats of either sex weighing between 150-200 grams were divided into 12 groups of 6 animals each and they were numbered individually.
2. The animals were fasted for 24 hrs before administering the drug with water *ad libitum.*
   Group I was administered with only 2% v/v tween 80 solution which served as control.
   Group II was administered with 100 mg/kg body weight of ibuprofen suspension orally which served as a standard.
3. Group III to group XII were administered with test compounds respectively. The dose being 100 mg/kg body weight selected on the basis of the standard drug used.
4. All the animal tails were dipped into a beaker containing water maintained at 55±1°C and the time taken for the animals to flick the tail from the hot water completely is recorded at 15 minutes, 30 minutes, 1 hour, 2 hours and 3 hours respectively.
5. The percentage of protection in the control, standard and drug treated animals were recorded and calculated by using the formula.
   % Analgesic activity (PAA) = [1- (Rt/Rc)] X 100
   Where Rt and Rc are the reaction time in test and control respectively.

The analgesic activity of the sixteen chalcones (**2a-2p**) has been evaluated by using acetic acid induced writhing method using aspirin as the standard drug. The observed analgesic activity of chalcones and pyrazoline derivatives by writhing method is presented in Table 2 and Fig 5

Table 2. Analgesic activity of synthesized compounds (2a-2p)

Compound	No. of writhings (mean ±SEM)	% Inhibition
2a	12.87 ± 0.42	32.93**
2b	11.54± 0.32	13.72**
2c	19.26 ±1.28	28.12**
2d	25.39 ± 0.59	38.84***
2e	18.49 ± 0.28	48.92***
2f	20.12 ± 0.65	61.49***
2g	22.54 ± 0.98	70.39***
2h	14.48 ± 0.48	53.90***
2i	18.34 ± 0.40	32.85**
2j	19.43 ± 0.65	29.63**
2k	31.45± 0.74	16.48**
2l	19.41 ± 1.54	43.94***
2m	19.40 ± 1.10	68.65***
2o	09.43 ± 0.65	55.74***
2p	09.21 ± 0.56	67.62***
Aspirin	8.38 ± 0.94	75.29***

Values are expressed as mean ± SEM (n=5).
*p<0.05; **p<0.01; ***p<0.001 compared to controls. Students's r-test

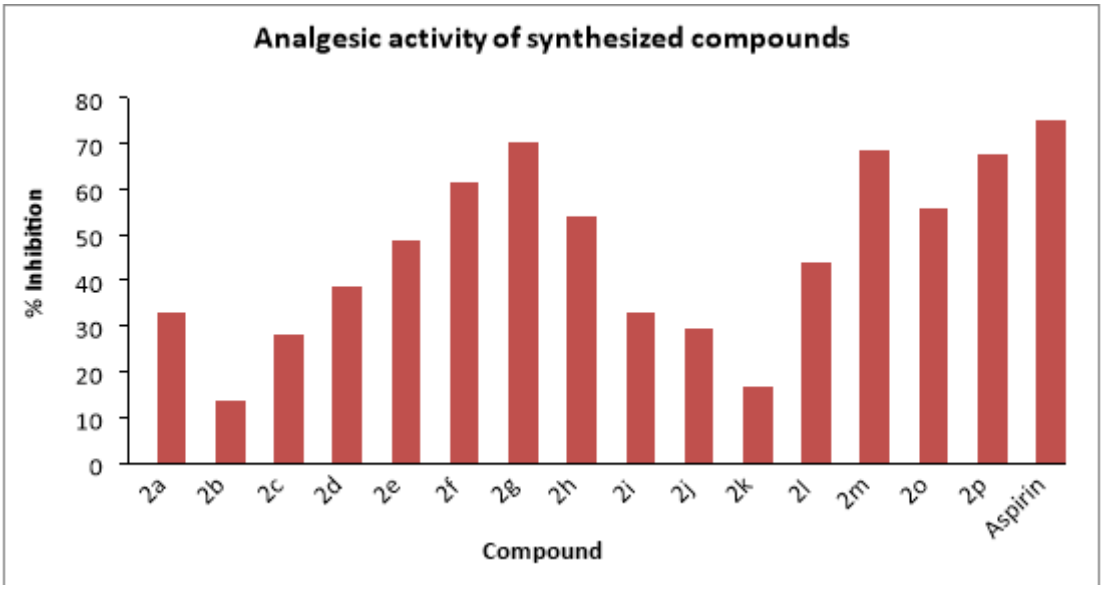


Fig. 5 Analgesic activity of synthesized compounds (2a-2p)

The synthesized compounds showed analgesic activity (percent inhibition) ranging from 16.48 to 70.39%. It was noted that compounds **2a, 2b, 2h, 2o** and **2p**

showed significant analgesic activity through out the test period. The activity of compound **2o and 2p** are very much comparable to that of standard reference drug aspirin. It indicates that they are effective against acetic acid induced writhing model.

Conclusion

The analgesic activity of the sixteen chalcones (**2a-2p**) has been evaluated by using acetic acid induced writhing method using aspirin as the standard drug. The observed analgesic activity of chalcones and pyrazoline derivatives by writhing method is presented in Table 2 and Fig 5.

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