IJPSR (2019), Volume 10, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 November 2018; received in revised form, 14 January 2019; accepted, 18 January 2019; published 01 July 2019

SYNTHESIS OF POTENTIAL ANTI-INFLAMMATORY PYRAZOLINE DERIVATIVES UNDER ULTRASONIC IRRADIATION

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Keywords:

Ultrasound irradiation, Chalcone, Pyrazole, Anti-inflammatory activity

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ABSTRACT: In the present investigation, the chalcone intermediates are synthesized by Claisen-Schmidt condensation reaction between acetanilide and appropriate aromatic aldehydes. Further, these chalcone intermediates were cyclized with phenylhydrazine in glacial acetic acid to give new pyrazolines derivatives using ultrasonic irradiation with higher yields in lesser time. All the synthesized derivatives are characterized by their TLC, Physical constant, FTIR, and ^{1}H NMR. The Chalcone intermediates were screened for their antimicrobial activity while cyclized pyrazolines derivatives were screened in search of potential anti-inflammatory compounds. The compound 4c had shown good potential for anti-inflammatory activity.

INTRODUCTION: Chalcones are *α, β*-unsaturated ketones which contain two aromatic groups and bridged by an enone linkage. They form the central core for a variety of important biological compounds. They are mainly intermediates in the synthesis of various biologically active compounds [1, 2]. The modifications on the chalcone backbone result in the generation of various heterocyclic systems. The acetamido group containing compounds have been found as essential pharmacophore, and thus incorporation of these in chalcone backbone may result in compounds with significant activity [3, 4]. When synthesized chalcones are subsequently cyclized with hydrazines, gives Pyrazolines [5, 6]. Among Pyrazoles, 2-pyrazolines are widely used as useful synthons in organic synthesis and having various biological activities [7, 8].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(7).3290-94

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(7).3290-94

Most of the synthetic methods for 2-pyrazolines suffer from several disadvantages such as long reaction times, expensive reagents, drastic reaction conditions, low yields, tedious workup procedures, and formation of byproducts. Nowadays, the application of Ultrasonic irradiation has aroused more and more interest in synthetic chemistry, which overcome the disadvantages and accelerates the reactivity millions fold and many synthetically useful reactions were accomplished [9-11].

Given this, the present investigation involves the synthesis of Pyrazoline derivatives through cyclization of synthesized chalcones having acetamido pharmacophore under ultrasonic irradiation in search of potential anti-inflammatory compounds [12-15].

MATERIALS AND METHODS: The starting materials and solvents used for each reaction are of synthetic grade procured from Sigma Aldrich. All the chemicals and synthesized products were characterized for their purity by physical constant and Thin Layer Chromatography (TLC). All the reactions were monitored by using thin layer

chromatography on pre-coated TLC plates (Silica gel 60-120#) by using solvent system Benzene: Ethanol [8:2] for Step-1 compounds and Dichloromethane: Ethyl acetate [9:1] for Step-2 compounds. The obtained TLC plates were observed under a long UV lamp in a UV chamber and also in iodine chamber for detection of spots. All reactions were carried out ultrasonic irradiation by using sonicator made of Labman Scientific Instruments. The synthesized compounds were characterized by spectroscopic method FTIR and ^{1}H NMR for elucidation and confirmation of their structures.

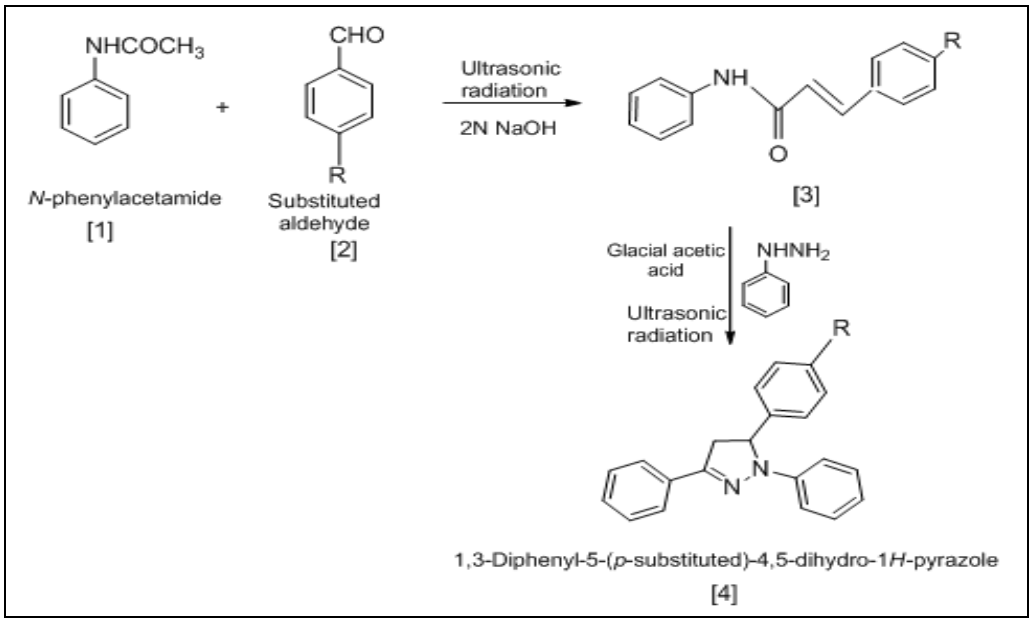
Synthesis Under Ultrasonic Irradiation:

Step I: Synthesis of Chalcones: Acetanilide (1, 1 mmole), dissolved with appropriate aromatic aldehydes (2, 1 mmole) in 95% of ethanol solvent (10 mL) and 2N sodium hydroxide (3 mL), taken into a 100 ml conical flask. The mixture was irradiated by an ultrasonic generator in a water-bath at 40-45 °C for 20 min. The reaction mixture so formed was diluted with water and neutralized with 2*N* hydrochloric acid. Collect the precipitate by filtration and washed well with cold water (2 × 20 mL). Further, recrystallization of crude product was carried out by using ethanol and collect the yellow crystals of chalcone 3a-e. Their physical characteristics data of these are reported in **Table 1**.

Step II: Synthesis of Pyrazolines: Synthesized chalcones 3a-e (1 mmole), phenylhydrazine, (2 mmoles) and glacial acetic acid (10 mL) were taken into a 100 mL conical flask. This reaction flask was suspended in the ultrasonic bath to get the ultrasound energy and sonicated until complete disappearance of chalcone. Further, the reaction mixture was poured into crushed ice and keep overnight. The obtained precipitate was collected by filtration and washed well with cold water (2 × 20 mL). Purification of crude product was carried out by recrystallization by using ethanol to give crystals of pyrazolines 4a-e. The products were characterized by IR and ^{1}H NMR spectral data. The physical characteristics data of these are reported in **Table 2**.

The following sequence of reaction appears to afford a satisfactory explanation of the mode of formation of the products. This reaction involves the initial formation of aryl hydrazones with the subsequent attack of nitrogen upon the carbon-carbon double bond.

Reaction Scheme:



Biological Activity:

Antimicrobial Activity of Chalcone: The antibacterial activity of all the synthesized compounds 3a-e were screened against different gram-positive (*Staphylococcus aureus* and *Streptococcus pyogenes)* and gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria by measuring zone of inhibition. The

antibacterial activity was performed by Agar diffusion method at the concentration level of 50 µg/ml. Ciprofloxacin was used as a standard drug at a concentration of 50 µg/ml. Nutrient agar was used as culture media, and DMSO was used as solvent [16-18].

Anti-inflammatory Activity of Pyrazolines: The anti-inflammatory of the test compounds 4a-e was carried out using the carrageenan-induced rat paw edema inhibition method. Acute inflammation was produced by subplantar injection of 0.1 ml of 1% suspension of carrageenan in the right hind paw of the rats, 30 min after oral administration of the drugs. The paw volume was measured plethysmometrically (IITC Digital Plethysmograph IITC-520) at 1, 2, 3, and 4 h after the carrageenan injection. Ibuprofen was used as the standard drug at a dose level of 10 mg/kg. The percentage inhibition of edema was calculated using the formula [19, 20].

$$\% \text{ Inhibition} = (1 - Vt/Vc) \times 100$$

Where *V*t is edema volume in treated groups, and *V*c is edema volume in control groups.

RESULTS AND DISCUSSION: All chemicals and products have shown single spot on TLC plate when observed under UV light and iodine chamber. The melting points were taken in open capillaries on melting point apparatus and were found uncorrected.

TABLE 1: PHYSICAL CHARACTERISTICS DATA OF SYNTHESIZED CHALCONE INTERMEDIATES

Comp. No.	R	Temp. (ºC)	Time (Min.)	% Yield
3a	$-NO_2$	30	30	84
3b	-Cl	25	40	81
3c	-F	25	40	78
3d	$-CH_3$	40	35	80
3e	-OH	35	30	86

TABLE 2: PHYSICAL CHARACTERISTICS DATA OF SYNTHESIZED PYRAZOLINE DERIVATIVES

Comp. No.	R	Temp. (ºC)	Time (Min.)	% Yield
4a	$-NO_2$	35	40	77
4b	-Cl	40	30	72
4c	-F	40	35	69
4d	$-CH_3$	35	40	75
4e	-OH	40	30	70

Spectral Data: The Infrared spectroscopy was carried out by using potassium bromide (KBr) pellet method on the Shimadzu IR Affinity-1. The NMR spectra were recorded on Bruker Avance III 500 MHz multi-nuclei solution NMR spectrometer. The characterization with IR and ^{1}H NMR spectra of the synthesized compounds confirmed the anticipated structure. The spectral data of synthesized pyrazolines has been shown in **Table 3**.

TABLE 3: SPECTRAL DATA OF SYNTHESIZED PYRAZOLINES

Compound 4a: 1,3-diphenyl-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole: M.P: 146 ºC **R_f:** 0.8		
IR (KBr cm-$^{1)}$	:	3211 (C–H), 1624 (C=N), 1332 (C–N), 1538(C=C), 1358 (NO_2)
Compound 4b: 1,3-diphenyl-5-(4-chloroophenyl)-4,5-dihydro-1H-pyrazole: M.P:182 ºC **R_f:** 0.7		
IR (KBr cm-$^{1)}$	:	3216 (C–H), 1640 (C=N), 1326 (C–N), 1542 (C=C), 710 (C–Cl)
^{1}H NMR (δ ppm)	:	3.109 (dd, 1H), 3.814 (dd, 1H), 5.210 (dd, 1H), 6.912–7.808 (m, 12H, Ar–H)
Compound 4c: 1,3-diphenyl-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazole: M.P:160 ºC **R_f:**0.6		
IR (KBr cm-$^{1)}$	:	3248 (C–H), 1637 (C=N), 1328 (C–N), 1568 (C=C), 1212 (C–F)
^{1}H NMR (δ ppm)	:	3.126 (dd, 1H), 3.832 (dd, 1H),5.224 (dd, 1H), 6.908-7.823 (m, 12H, Ar–H)
Compound 4d: 1,3-diphenyl-5-(4-methylphenyl)-4,5-dihydro-1H-pyrazole: M.P:156 ºC **R_f:**0.8		
IR (KBr cm-$^{1)}$	:	3256 (C–H), 1629 (C=N), 1364 (C–N), 1554 (C=C)
Compound 4e:1,3-diphenyl-5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazole: M.P:134 ºC **R_f:**0.7		
IR (KBr cm-$^{1)}$	:	3248 (C–H), 1637 (C=N), 1328 (C–N), 1568 (C=C), 3542 (OH)

Biological Activity: Though we have many synthetic drugs in the market, the bacterial mutations are making them resistance. Given this, the synthesized intermediate compounds in the present investigation (3a-e) were evaluated for their antimicrobial activity.

Antimicrobial Activity of Chalcones: It is observed that the chalcone derivatives 3a-e showed antibacterial activity. From the result, It has been observed that the compound 3b and 3c showed good potential similar to the standard. In general, it is worth noting that compounds having fluorine and chlorine group in the scaffold exhibited excellent activity while the compounds having other substituent showed good to moderate activity. Antibacterial activity data (Zone of inhibition) of the synthesized chalcone derivatives 3a-e are presented in **Table 4**.

TABLE 4: ANTIMICROBIAL ACTIVITY OF COMPOUNDS 3a-e

Compound Code	Compound	Zone of inhibition (mm)			
		Gram positive bacteria		Gram negative bacteria	
		S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*
3a	NO_2	8	10	12	14
3b	Cl	9	10	13	14
3c	F	10	11	15	17
3d	CH_3	10	10	11	13
3e	OH	9	10	13	15
Standard	Ciprofloxacin	13	14	18	19
Solvent	DMSO	-	-	-	-

Anti-inflammatory Activity of Pyrazolines: Assessment of anti-inflammatory action was completed *via* carrageenan-induced rat paw edema inhibition method. The rate of edema hindrance was computed from the mean impact in control and treated creatures agreeing on the accompanying condition. Anti-inflammatory activity data of the synthesized Pyrazoline derivatives 4a-e are presented in **Table 5**.

TABLE 5: ANTI-INFLAMMATORY ACTIVITY OF PYRAZOLINES

Compound	Mean paw Oedema Volume (ml) ± SE						
	0 hr	½ h	1h	2 h	3 h	4 h	% inhibition after 4 h
Control	0.122±0.0502	0.411±0.0749	2.14±0.163	2.84±0.279	2.60±0.318	1.42±0.314***	--
Drug	0.0826±0.0762	0.615±0.237	1.12±0.236	1.54±0.178	1.24±0.271	0.956±0.182***	51
4a	0.124±0.0462	0.356±0.0823	1.55±0.432	1.86±0.139	1.42±0.326	1.29±0.243***	32
4b	0.142±0.0514	0.339±0.0627	1.38±0.210	1.59±0.130	1.30±0.318	1.27±0.128***	36
4c	0.110±0.0420	0.358±0.0524	1.43±0.136	1.58±0.216	1.42±0.114	1.26±0.135***	39
4d	0.124±0.0414	0.255±0.129	1.39±0.182	1.67±0.232	1.34±0.428	1.26±0.224***	34
4e	0.161±0.0429	0.242±0.109	1.78±0.136	1.52±0.249	1.41±0.132	1.21±0.132***	34

Values are expressed as mean ± SEM of six animals in each group. *Statistically significant ($P \leq 0.05$). **Statistically significant ($P \leq 0.001$)

CONCLUSION: Some of the new Pyrazoline derivatives were synthesized under ultrasonic irradiation and characterized by various analytical methods. These are successfully evaluated for their anti-inflammatory activity. Spectral data confirm the structure of the synthesized pyrazolines as expected. From the results, it can be concluded that the modified pyrazolines shows remarkable anti-inflammatory action and having the potential to study further.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: No conflict of interest.

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How to cite this article:
Sethiya JP, Bhavsar SP and Shahare HV: Synthesis of potential anti-inflammatory pyrazoline derivatives under ultrasonic irradiation. Int J Pharm Sci & Res 2019; 10(7): 3290-94. doi: 10.13040/IJPSR.0975-8232.10(7).3290-94.