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SYNTHESIS, CHARACTERIZATION, ANTI MICROBIAL, ANALGESIC ACTIVITIES OF 3-(3-CHLOROPHENYL)-5-PHENYL-4,5-DIHYDRO-1,2-OXAZOLE DERIVATIVES

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ABSTRACT
Novel Isoxazoles are prepared by treating chalcone derivatives from 3- Chloro acetophenone with hydroxyl amine hydro chloride, small amount of sodium acetate and ethanol. The chalcone were prepared by using the 3-chloro Acetophenone with aromatic aldehydes in presence of NaOH followed by Clasein-Schmidt condensation reaction. All the synthesised compounds were characterized by using the IR,H^1 NMR spectroscopy. The biological evolution like anti bacterial and analgesic activity was performed for test compounds to identify the activity. Here the disc diffusion method performed to identify the anti bacterial activity by measuring of zone of inhibition in mm compared with standard streptomycin and analgesic activity by performing the tail immersion method measuring the percentage of inhibition compare with standard Paracetamol at dose of 100 mg/kg.

KEY WORDS: chalcones. Anti bacterial, analgesic, Tail immersion method, disc diffusion method.

INTRODUCTION

Heterocyclic compounds are abundant in nature and are of great significance to life because their structural subunits exist in many natural products such as vitamins, hormones, and antibiotics[1,2]. Hence, they have attracted considerable attention in the design of biologically active molecules[3,4] and advanced organic chemistry[5,6]. Also in the family of heterocyclic compounds nitrogen containing Heterocyclic compounds are an important in the medicinal chemistry and also contributed to the society from biological and industrial point which helps to understand life processes[7].

Isoxazoline are is the unique molecules possessing the oxygen and nitrogen in the five member ring at 1,2 positions[8]. The nucleus usefulness in drugs designing different types of diseases due it's wide range of pharmacological activities . It has been reported that isoxazolines possess analgesic, anti-inflammatory[9-12] and antimicrobial[13-19]. Isoxazoline containing different drugs are available in market.

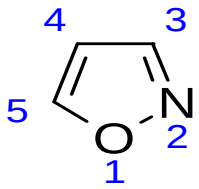


Fig:1 isoxazole structure.

Experimental work

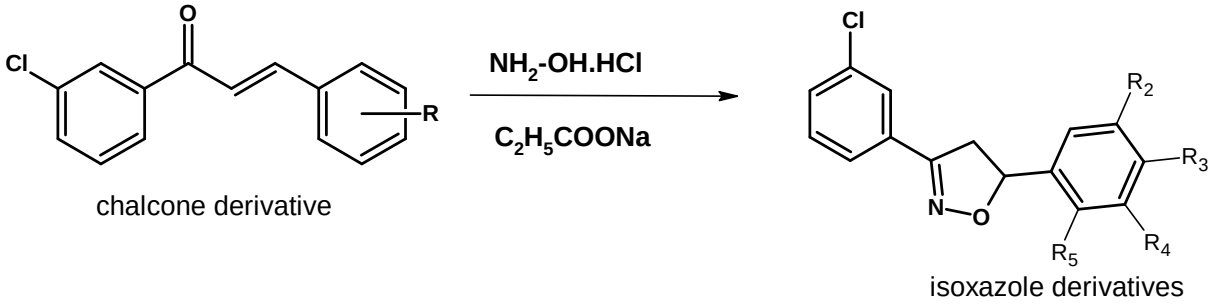
MATERIALS AND METHODS

(2*E*)-1-(3-chlorophenyl)-3-phenylprop-2-en-1-one derivatives, hydroxyl amine, sodium acetate, glacial acetic acid, conc. HCl, DMSO, DPPH reagent .all the reagents were purchased analytical grade. Melting points were determined on a capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded in the indicated solvent on Bruker WM 400 MHz spectrometer with TMS as internal standard. Infrared spectra were recorded in KBr on Perkin-Elmer AC-1 spectrophotometer. Column chromatography was performed on silica gel (Merck, 60-120 mesh).

General procedure for the synthesis Isoxazolines[20-21]

A mixture of (2*E*)-1-(3-chlorophenyl)-3-phenylprop-2-en-1-one derivatives (0.02 mol), Hydroxyl amine hydrochloride (0.02 mol) and catalytic amount of sodium acetate in ethanol (25 ml) was refluxed for 6 h. The mixture was concentrated by distilling out the solvent under reduced pressure and poured into ice water. The precipitate obtained was filtered, washed and recrystallized from ethanol. The completion of the reaction was monitored by TLC. Similarly various isoxazole derivatives I2-5were prepared.

Scheme of preparation



chalcone derivative

isoxazole derivatives

Table: 1 List of aldehydes.

S. No	R_2	R_3	R_4	R_5
1.	H	H	H	H
2.	H	H	OH	H
3.	H	H	Cl	H
4.	H	H	S-CH_3	H
5.	H	OCH_3	OCH_3	H

Biological evolution of compounds

Based on the literature, chalcones were reported to possess antimicrobial activity, anti oxidant, anti inflammatory, analgesic, anti cancerous, etc. Therefore the present work performs the anti microbial, anti oxidant activities.

Antibacterial activity[22-23]

The antibacterial activity was tested by determining inhibitory concentration by diffusion disc technique. The bacterial strains were obtained from National Chemical Laboratories (NCL), Pune and Microbial Type Culture Collection (MTCC), Chandigarh. The strains used for the present study were Staphylococcus aureus (MTCC 737) Bacillus subtilis (MTCC 441), Escherichia coli (MTCC 1687), P.vulgaris MTCC 1771.

Procedure

The antimicrobial activity of the compounds was assessed by disc diffusion method Nutrient agar medium was prepared and sterilized by an autoclave. In an aseptic room, they were poured into a petridishes to a uniform depth of 4 mm and then allowed to solidify at room temperature .After solidification, the test organisms, Staphylococcus aureus, Bacillus subtilis, , Escherichia coli and P. vulgaris were spread over the media with the help of a sterile swab socked in bacterium and is used for antibacterial study. The synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) to produce a concentration of 500 µg/disc ,1 mg/disc and used for the study. Streptomycin 5 µg/disc was used as the standard. Then the sterile filter paper discs (6mm) having a capacity to hold 10 µl of solution were immersed in definite concentration of compounds and placed over the solidified agar in such a way that there is no overlapping of the zone of inhibition. Plates were kept at room temperature for half an hour for the diffusion of the sample into the agar media. The organism inoculated petridishes were incubated at 37 °C for 24 hours. After the incubation period is over, the zone of inhibition produced by the samples and standard were measured. All tests were performed in triplicate. The results were tabulated in Table-4.

Analgesic activity[24]

The analgesic activity was determined by tail immersion method.[12] Wistar albino mice (n = 6) of either sex selected by random sampling technique were used for the study. Paracetamol at (100 mg/kg) was administered as standard drug for comparison. The test compounds (100 mg/kg) were administered orally by intragastric tube. The animals were held in position by a suitable restrained with the tail extending out and the tail (up to 5 cm) was then dipped in a beaker of water maintained at 55 ± 5 ^{0}C. The time in seconds taken to withdraw the tail clearly out of water was taken as the reaction time. The reading was recorded at 30, 60, 120 and 180 min after administration of compounds. A cut off point of 10 sec was observed to prevent the tail damage. The results were tabulated in Table-5.

RESULTS AND DISCUSSION

Table2: Physical Data.

Compound	R	Molecular Formula	Relative Molecular Mass (RMM)	Melting Point (^{o}C)	Yield %
I-1	H	$C_{15}H_{12}ClNO$	257.7	125	89
I-2	OH	$C_{15}H_{12}ClNO_2$	273.7	115	92
I-3	Cl	$C_{15}H_{11}Cl_2NO$	292.1	122	91
I-4	S-CH_3	$C_{16}H_{14}ClNOS$	303.8	105	94
I-5	DI O-CH_3	$C_{17}H_{16}ClNO_3$	317.7	114	85

Table 3: Elemental composition

Compound	%Calculated				%Found			
	C	H	CL	O	C	H	Cl	O
I-1	69.91	4.69	13.76	6.21	69.85	4.45	13.55	6.01
I-2	65.82	4.42	12.95	11.69	65.75	4.36	12.85	11.69
I-3	61.67	3.79	24.27	5.48	61.56	3.79	24.25	5.38
I-4	63.25	4.64	11.67	5.27	63.22	4.56	11.56	5.15
I-5	64.26	5.08	11.16	15.10	64.22	5.00	11.05	15.06

Spectral data

3-(3-chlorophenyl)-5-phenyl-4,5-dihydro-1,2-oxazole *(I-1)*

IR(cm-1) 1794.24 (C=O), 1591(C=N), 1097(C-N);3667.07 (C-Cl), 1450 (C=C); 3.366 (1H, s, -C-Cl) 6.0-9.1 (1H, m, Ar-H), 7.2-8.4 (5H, s, Ar-OH)

4-[3-(3-chlorophenyl)-4,5-dihydro-1,2-oxazol-5-yl]phenol *(I-2)*

IR(cm-1) 1794.24 (C=O), 1591(C=N), 1097(C-N);667.07 (C-Cl), 1450 (C=C), 3400 (Ar-OH), 3.366 (1H, s, -C-Cl), 6.0-9.1 (1H, m, Ar-H), 7.2-8.4 (5H, s, Ar-OH)

3-(3-chlorophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1,2-oxazole *(I-3)*

IR(cm-1) 1794.24 (C=O), 1591(C=N), 1097(C-N);667.07 (C-Cl), 1450 (C=C), 3.366 (1H, s, -C-Cl), **6.0-9.1 (1H, m,** Ar-H), 7.2-8.4 (5H, s, Ar-OH)

3-(3-chlorophenyl)-5-[4-(methylsulfanyl) phenyl]-4,5-dihydro-1,2-oxazole *(I-4)*

IR(cm-1) 1794.24 (C=O), 1591(C=N), 1097(C-N);667.07 (C-Cl), 1450 (C=C), 3.366 (1H, s, -C-Cl), 6.0-9.1 (1H, m, Ar-H), 7.2-8.4 (5H, s, Ar-OH)

3-(3-chlorophenyl)-5-(3,4-dimethoxyphenyl) -4,5-dihydro-1,2-oxazole *(I-5)*

IR(cm-1) 2568.21 (C-S), 1591(C=N), 1097(C-N);, 667.07 (C-Cl), 1450 (C=C), 2860 (C-O-CH_3 P – 153 674.39 (C-Cl), 1614.81 (C=C), 3.366 (1H, s, -C- Cl), 6.0-9.1 (1H, m, Ar-H)

Anti bacterial evolution

Table4: anti bacterial evolution.

Compound code	S. aureus		*E.coli*		P. aeruginosa	
	Zone of inhibition (mm)					
	50 µg/ ml	100 µg/ ml	50 µg/ ml	100 µg/ ml	50 µg/ ml	100 µg/ ml
I-1	11	24	10	22	11	20
I-2	13	26	13	23	11	22
I-3	16	24	14	26	13	26
I-4	13	26	12	24	12	24
I-5	12	25	12	22	11	22
Streptomycin	18	34	16	32	16	32

Analgesic activity

Table5: Analgesic Results.

Compound	Dose	Percentage of analgesic activity			
Code	(mg/kg)	30 min.	1 hour	2 hour	3 hour
I-1	100	31 ± 0.38*	33 ± 0.72*	38 ± 0.47*	29± 0.91*
I-2	100	37± 0.28*	42± 0.45**	45± 0.49*	37 ± 0.26*
I-3	100	46 ± 0.22*	51 ± 0.22**	58 ± 0.43*	48 ± 0.27*
I-4	100	41 ± 0.52**	45± 0.23**	50 ± 0.21**	37± 0.29*
I-5	100	40 ± 0.26*	44 ±0.31**	48± 0.32**	37± 0.44**
Paracetamol	100	38± 0.42**	47± 0.82**	52± 0.71**	33 ± 0.31**

DISCUSSION

The above synthesized compounds anti microbial evolution were performed by using Diffusion method by the calculation of Zone of inhibition against the test organisms, the compounds shows that compound IS-06 shows maximum activity than compare with other compounds, the compound I-3 (3-(3-chlorophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1,2-oxazole) shows activity at concentration of Staphylococcus aureus zone of inhibition 16,24 mm at 50 µg/ml ,100 µg/ml with Pseudomonas vulgaris zone of inhibition 13,26 mm at 50 µg/ml ,100 µg/ml with Escherichia coli the zone of

inhibition 14,26 mm at 50 µg/ml ,100 µg/ml.. the compound I-4 (3-(3-chlorophenyl)-5-[4-(methylsulfanyl) phenyl]-4,5-dihydro-1,2-oxazole) shows activity at concentration of Staphylococcus aureus zone of inhibition 13,26 mm at 50 µg/ml ,100 µg/ml with Pseudomonas vulgaris zone of inhibition 12,24 mm at 50 µg/ml ,100 µg/ml with Escherichia coli the zone of inhibition 12,24 mm at 50 µg/ml ,100 µg/ml..

The analgesic activity the compound the compound I-3 (3-(3-chlorophenyl)-5-(4-chlorophenyl)-4, 5-dihydro-1, 2-oxazole) shows activity at 100 mg/kg dose 46 ± 0.22, 51 ± 0.22,58 ± 0.43, 48 ± 0.27 at time interval of 30 min,1 hour,2 hour,3 hour respectively.

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CONCLUSION

The above results we concluding the compound IS-04 was showing the better anti microbial activity against both gram positive and gram negative the organism. The reason is due that compound contain electron with drawing group than that of other compounds. In case of analgesic activity, compound IS-04 shows better anti oxidant than other compounds We concluding the compound IS-04 was be best fit molecule against microbes, anti-oxidant activity on the further exploration of the compound the statement may confirmed.

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