Evaluation of some novel chalcone derivatives for antimicrobial and anti-inflammatory activity

Neeraj kumar[1], Jain, J. S.[2*], Sinha, R.[2], Garg, V. K.[3], Bansal, S. K.[4]

[1] *Azad Institute of Pharmacy and Research, Lucknow, Uttar Pradesh, India*
[2]*Department of Pharmacy, Ram Eesh Institute of Vocational and Technical Education, Uttar Pradesh, India*
[3]*Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, Uttar Pradesh, India*
[4]*Bharat Institute of Technology, Uttar Pradesh, India*

Abstract

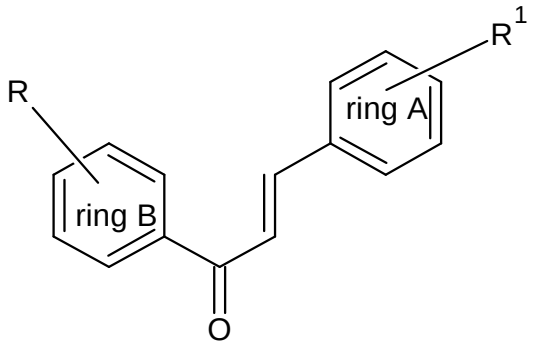
A series of chalcones of substituted acetophenone and substituted aryl aldehydes were synthesized and evaluated for antimicrobial and anti-inflammatory activity. The structures of synthesized compounds were confirmed by IR and 1H NMR spectroscopy. The antimicrobial activity was evaluated against *S. aureus, B. subtilis, E. coli* and *P. putida* strains whereas anti-inflammatory activity was performed by Carrageenan induced hind paw oedema method. Results obtained showed that 40% of the synthesized compounds exhibited significant antimicrobial activity against all the tested micro organisms. Compound N^1 i.e. 3-(4-nitrophenyl)-1-phenyl prop-2-en-1-one emerged as the most active compound with MIC_{50} value of 471µg/ml against *S. aureus*. In anti-inflammatory screening compound N^3, 3-(3,5-dimethoxy phenyl)-1-phenyl prop-2-en-1-one and compound N^6, 3-(4-hydroxy phenyl)-1-phenyl prop-2-en-1-one emerged as the most potent molecules with inhibition of 38.46% and 50.0% as compared to 42.30% of Diclofenac Sodium at 1.0 hr interval of the carrageenan induced oedema.

Key Words: Chalcone**,** Antimicrobial activity, Anti-inflammatory activity, Carrageenan.

Introduction

Chalcones are the aromatic ketones belonging to 1, 3-diaryl-2-propen-1-ones. Chalcones are also known as benzylideneacetophenones or phenyl styryl ketones. Naturally occurring chalcones belong to the flavanoids. Chemically, they consist of open chain flavonoids in which the two aromatic rings are joined by a three carbon α .β unsaturated carbonyl system. They show broad spectrum of biological activities like antibacterial [1-4]**,** antimycobacterial [5], antifungal [6],

antitumor, anthelmintic, amoebicidal, antiulcer, antiviral, insecticidal, antiprotozoal, anticancer, cytotoxic, immunosuppressive and anti inflammatory [7-10]. Chalcones also acts as intermediates in the biosynthesis of flavonoid that are substances wide spread in plants with biological activities. Chalcones are one of the major classes of natural products with widespread distribution in fruits vegetables, spices, tea & soya based foodstuff and variety of trees and plants. Chalcones exist as either *E* or *Z* isomers. *E* isomer is the most stable form and consequently majority of chalcones are isolated as *E* isomer [11]. In present study, ten chalcone derivatives of acetophenone, 4-amino acetophenone and 4-hydroxy acetophenone with substituted aromatic aldehydes (Compounds N^1 to N^{10}) were synthesized and evaluated for anti microbial and anti-inflammatory properties. Chalcones of 4-substituted amino acetophenone with large bulky groups in ring A have been reported to possess potent antibacterial activity against methicillin resistant S. aureus [12].



The most potent compound in this study had piperazine in the 2-position of ring A. Kromann et al have reported that free hydroxyl group in 4-position of ring B was necessary for antibacterial activity against S. aureus . However, hydroxyl group in ring A can be replaced without affecting the activity. Hence the present author has reported the synthesis and evaluation of antibacterial properties of chalcones of 4-amino acetophenone and 4-hydroxy acetophenone. Dimethyl amino chalcones are reported to possess potent anti-inflammatory properties. They had shown potent in vitro inhibitory activity on the production of NO and PGE_2 mediators produced by RAW 264.7 macrophage cells. Hence the present author also evaluated 4-amino substituted and other chalcones for anti-inflammatory activity.

Materials and Methods

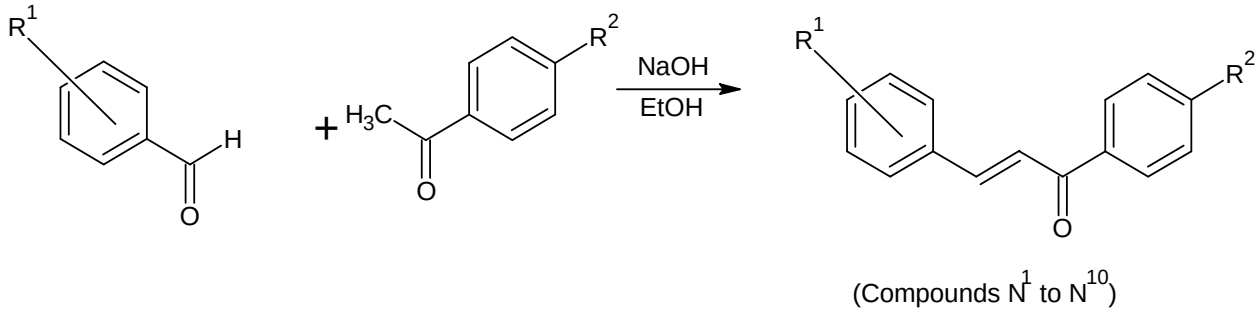
Chemistry

Melting points were determined by open capillary method and are uncorrected. The purity of compounds was confirmed by thin layer chromatography using Silica gel G as stationary phase and Chloroform: Methanol (9:1) as the mobile phase.The spots were visualized with iodine vapors. 1H NMR spectra were recorded in $CDCl_3$ on Brucker 300 MHz instrument. Chemical shift values are expressed in parts per million (ppm, δ). IR spectra were recorded in KBr disc on Shimadzu FTIR 8400 spectrophotometer.

Synthetic procedures for chalcones (Compound N^1 to N^{10})

Title compounds were synthesized according to *Scheme 1*. Sodium Hydroxide (2.2g, 0.056 mol) was dissolved in 20 ml of distilled water and stirred in ice cold conditions. Freshly distilled

acetophenone or substituted acetophenone (0.043 mol) was dissolved in 20 ml of 95% v/v ethanol and the solution was added drop wise with constant stirring under ice cold conditions.



Scheme I: Synthesis of chalcones (Compounds N^1 to N^{10})

Pure benzaldehyde or substituted benzaldehyde (0.046 mol) was dissolved in 20 ml of 95% v/v ethanol and added drop wise to the previous solution with constant stirring under ice cold conditions.

Table 1: Physico-chemical properties synthesized chalcones

Comp. No.	R^1	R^2	Mol. Formula	Mol. Wt.	Reaction Time (hrs)	% yield	m.p. (°C)	R_f [a]
N^1	4-NO_2	H	$C_{15}H_{11}NO_3$	253	26	64	157-59	0.46
N^2	2-OH	H	$C_{15}H_{12}O_2$	224	72	54	149-52	0.20
N^3	3,5-di OCH_3	H	$C_{17}H_{16}O_3$	268	48	58	80-2	0.32
N^4	4-Cl	H	$C_{15}H_{11}ClO$	242	30	67	110-12	0.57
N^5	4-Br	H	$C_{15}H_{11}NO_3$	287	46	52	115-17	0.27
N^6	4-OH	H	$C_{15}H_{11}BrO$	224	72	59	173-75	0.81
N^7	4-Br	NH_2	$C_{15}H_{12}BrNO$	302	60	70	150-52	0.50
N^8	4-Cl	NH_2	$C_{15}H_{11}NO_3$	257	52	78	85-7	0.47
N^9	4-Cl	OH	$C_{15}H_{12}ClNO$	258	70	57	195-97	0.23
N^{10}	4-dimethyl amino	H	$C_{17}H_{17}NO$	251	65	60	110-12	0.59

[a] Mobile phase in TLC: Chloroform: Methanol (9:1)

The stirring was continued till the TLC had shown the disappearance of aldehyde spot (stirring time specified in Table 1). pH of the reaction mixture was made neutral by addition of dil. HCl. The product was filtered under vacuum, washed with excess distilled water and recrystallized from organic solvent [absolute alcohol, Petroleum ether:chloroform (1:1) and chlorpform:ethanol (3:1)]. Physico chemical data of synthesized compounds is specified in Table 1 whereas IR and 1H NMR data are specified in Table 2.

Table 2: Spectral (IR and 1H NMR) data of synthesized compounds

Comp. No.	IR data (cm^{-1})	1H NMR data (δ, ppm)
N^1	3076, 2923, 1700, 1658, 1550,1300, 1218	8.28 (d, 2H, J=8.68), 8.04 (d, 2H, J=7.24), 7.86-7.78 (m, 3H, ArH), 7.68-7.66 (br s, 1H), 7.64-7.61 (m, 1H), 7.57-7.52 (m, 2H, ArH).
N^2	3211, 3080, 2950, 1700, 1639, 1230	8.11-8.09 (m,1H), 8.08-8.045(m, 1H), 7.88-7.85(m,2H), 7.84-7.82 (m,1H), 7.64 (d, 1H, J=7.9), 7.59-7.54 (m,2H, ArH), 7.30-7.25 (m, 2H, ArH), 6.99-6.88 (m, 2H, ArH).
N^3	3075, 2957, 1700, 1650, 1340	8.02-7.99 (d, 2H, J=6.83), 7.73(s, 1H, ArH), 7.55-7.33 (m, 3H, ArH), 7.28 (br s, 1H, ArH), 7.22-7.15 (m, 2H, ArH), 6.88-6.77 (m, 1H, ArH), 4.35-3.35 (br s, 6H).
N^4	3056, 2923, 1700, 1658, 810	8.00 (d, 2H, J= 6.92), 7.82-7.720 (s, 1H, ArH), 7.71 -7.47 (m, 5H, ArH), 7.31 (d, 3H, ArH).
N^5	3055, 2956, 1700, 1658, 979	8.06 (d, 2H, J=6.72), 7.83-7.52 (m, 1H, ArH), 7.78-7.25 (m, 2H, ArH), 7.64 (s, 1H, ArH), 7.57 (m,3H, ArH), 7.46-7.35 (m, 2H,ArH).
N^6	3224, 3064, 2950, 1700, 1654, 1200	7.89-7.84 (m,3H), 7.79-7.77 (m, 3H, ArH), 7.52 -7.48 (m, 2H, ArH), 7.41 -7.38 (m, 2H, ArH), 6.62 (d, 2H, ArH, J=7.872)
N^7	3300, 3053, 2871, 1700, 1650, 1220	7.76 (d, 2H, ArH, J=8.53), 7.61(d, 1H, J=6.93), 7.48 (d, 1H, J=7.66), 7.34 (m, 4H, ArH), 7.25-7.20 (m, 2H, ArH), 6.56 (m, 2H, ArH)
N^8	3309, 3053, 2925, 1700, 1645, 1263, 815	7.78-7.75 (m, 2H, CH), 7.63-7.59 (m, 4H, CH and ArH), 7.31-7.19 (m, 4H,ArH), 6.63 (d, 2H, NH_2)
N^9	3379, 3053, 2930, 1720, 1643, 1226, 813	7.85 (d, 2H, CH, J=8.4), 7.825 (s, 1H, ArH), 7.78-7.75 (m, 3H, ArH), 7.51 (s,1H,ArH), 7.466 (s,1H, ArH), 7.384 (d, 2H, ArH, J=8.4), 6.623 (br s, 1H, ArOH)
N^{10}	3053, 2910, 1700, 1649, 1226	8.002 (d, 2H, CH, J=7.68), 7.831 (s,1H, ArH), 7.779(s, 1H, ArH), 7.57 -7.48 (m, 5H, ArH), 7.372 (s, 1H, ArH), 7.320 (s,1H, ArH), 6.72-6.69 (s, 6H, di CH_3)

Biological Evaluation

All synthesized compounds were evaluated for antimicrobial and anti-inflammatory properties.

Antimicrobial activity

Compounds N^1 to N^{10} were evaluated for antimicrobial activity against four different strains (two gram +ve viz. *S. aureus* and *B. subtilis* and two gram –ve viz. *E. coli* and *P. putida*) by agar disc diffusion method. The results are compared with standard antibiotics like Kanamycin, Chloramphenicol, Penicillin G and Ampicillin. The details of results of zone of inhibition of standard antibiotics and test compounds are given in **Table 3** and **Table 4**. Compounds N^1, N^5, N^6 and N^9 were further screened for MIC_{50} value determination. The details are specified in **Table 5**.

Table 3: Antibacterial activity of standard antibiotics

Standard Antibiotic	**Conc. (µg/ml)**	**Zone of Inhibition (mm)**			
		S. aureus	*B. subtilis*	*E. coli*	*P. putida*
Kanamycin	100	1.7	1.3	1.2	1.3
Chloramphenicol	100	1.8	1.1	1.0	0.9
Penicillin G	100	0.6	0.8	0.8	0.5
Ampicillin	100	1.0	1.0	1.0	0.9

Table 4: Antibacterial activity of synthesized compounds (N^1 to N^{10})

Comp. No.	Antimicrbial activity			
	S. aureus	B. subtilis	E. coli	P. putida
N^1	++++	+	++++	+
N^2	----	+	++++	+
N^3	----	----	+	----
N^4	----	----	----	----
N^5	++++	++	++	++++
N^6	++++	++	++++	++
N^7	----	----	----	----
N^8	++++	+	----	----
N^9	++++	+	----	++++
N^{10}	----	++	----	----

++ Moderate activity, ++++ Strong activity, ---- No activity

Table 5: MIC_{50} determination of synthesized compounds (N^1, N^5, N^6 and N^9)

Comp. No.	MIC_{50} readings (µg/ml)			
	S. aureus	B. subtilis	E. coli	P. putida
N^1	471	-	986	-
N^5	965	-	-	894
N^6	1240	-	1100	-
N^9	916	-	-	843

(-) indicates no activity

Anti-inflammatory activity

All synthesized compounds were evaluated for anti-inflammatory properties by Carrageenan induced hind paw edema method [13]. The institutional animal ethics committee (IAEC) approved the use of animals for the present study, **(Ethical clearance number: 711/02/a/CPCSEA).** Male albino rats (125-130g) were used as experimental animals. Compounds N^1 to N^{10} were administered orally to rats in the dose of 200mg/kg and the percent edema inhibition was determined at 1h, 2h and 3h duration. The results are compared with standard anti-inflammatory drug, Diclofenac Sodium (50mg/kg). The data are analyzed statistically at 95% probability scale. The details are given in **Table 6.**

Table 6: Anti-inflammatory activity of synthesized compounds

Sr. No	**Oedema volume (Mean ±S.E)**			**Oedema inhibition (%)**		
	1h	2h	3h	1h	2h	3h
Control	0.266±0.024	0.33± 0.4	0.30± 0.035	-	-	-
Diclofenac Sodium	0.15±.0.01	0.20±0.23	0.17± 0.019	42.30	39.33	43.33
N_1	0.25±0.030	0.31± 0.03	0.31± 0.03	3.84	6.06	10.00
N_2	0.21±0.030	0.32± 0.021	0.32 ± 0.021	19.23	3.03	3.33
N_3	0.16±0.022	0.23 ± 0.01	0.23 ±0.01	38.46	30.30	13.33
N_4	0.24±0.01	0.29± 0.15	0.29±0.01	7.69	12.12	16.66
N_5	0.21± 0.03	0.25 ±0.01	0.25±0.018	19.23	24.24	23.33
N_6	0.13± 0.02	0.21± 0.034	0.21±0.034	50.00	36.36	3.33
N_7	0.25 ±0.02	0.38± 0.04	0.38 ± 0.04	3.84	6.06	3.33
N_8	0.21±0.060	0.28 ± 0.03	0.28±0.03	19.23	15.15	3.33
N_9	0..25±0.018	0.40±0.049	0.40 ±0.049	3.84	3.03	16.66
N_{10}	0.21± 0.01	0.23± 0.03	0.23± 0.03	19.23	30.30	36.66

Results and Discussion

The results of antimicrobial studies have shown that four compounds out of 10 viz. N^1, N^5, N^6 and N^9 were found to possess antimicrobial activity against all the tested micro-organisms. Compound N^2 has shown antimicrobial activity against *E.coli* only whereas compound N^8 was found to be active against *S.aureus* only. Compounds N^1, N^5, N^6 and N^9 were further subjected to MIC_{50} value determination. Compounds N^1 and N^6 were evaluated against *E.coli* and *S.aureus* whereas compound N^5 and N^9 were tested against *P.putida* and *S.aureus* respectively. The results have shown that chalcones possess mild antibacterial activity against the four tested strains. Only one compound N^1 i.e. chalcone of 4-nitrobenzaldehyde with acetophenone has shown MIC50 value of 471ug/ml against *E.coli*. Compounds N^1 to N^{10} were also evaluated for anti-inflammatory activity in albino rats by Carrageenan induced hind paw edema method. Results obtained have shown that three compounds N^3, N^6 and N^{10} possess significant anti-inflammatory effects. Compound N^6, a chalcone of 4-Hydroxy benzaldehyde with acetophenone was found to be the most active compound and its effects are more potent than the standard anti-inflammatory drug, Diclofenac Sodium. It has shown 50% inhibition of edema as compared to 42.30% of Diclofenac Sodium at 1.0h duration in the dose of 200mg/kg. However the effect was diminished to 3.33% at 3.0h duration, indicating shorter duration of action. Compound N10, a chalcone of 4-dimethyl amino benzaldehyde with acetophenone have shown increase in % edema inhibition from 19.23% at 1.0h to 36.66% at 3.0h interval, indicating longer duration of action. Thus compounds N^6 and N^{10} could be explored further for development of a potent anti-inflammatory agent.

Acknowledgements

Authors are highly thankful to Dr. G. T. Kulkarni, Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology for providing the infrastructure for the project work. Authors deeply acknowledge the contribution of Dr. Seema Sangwan, Department of Biotechnology, MIET, for carrying out the antimicrobial evaluation. Authors are also thankful to Mr. M. N. Lal, Indian Institute of Technology, New Delhi for spectral analysis of the compounds.

References

1. D.N. Dhar In Chemistry of chalcones and related compounds, 1st ed., John Wiley, New York, **1981**: pp.
2. RI Tsukiyama; H Katsura; N Tokuriki; M Kobayashi. *Antimicrob Agent Chemother*, **2002**, 46, 1226.
3. H Kromann; M Larsen; T Boesen; K Schonning; SF Nielson. *Eur J Med Chem*, **2004**, 39, 993.
4. SF Nielson; M Larsen; T Boesen; K Schonning; H Kromann. *J Med Chem*, **2005**, 48, 2667.
5. SF Nielson; T Boesen; M Larsen; K Schonning; H Kromann. *Bioorg Med Chem*, **2004**, 12, 3047
6. S Lopez; MV Castelly; S Zacchino; JN Dominguez; G Lobo; J Charris; JC Coetres; JC Ribas; C Devia; AM Rodrigues; RD Enriz. *Bioorg Med Chem*, **2001**, 9, 1999.

7. F Herenencia; ML Fernandiz; A Ubeda; JN Dominguez; JE Charris; GM Lobo; MJ Alkaraj. *Bioorg Med Chem Lett*, **1998**, 8, 1169.
8. F Herencia; ML Fernandiz; A Udeba; I Guillen; JN Dominguez; JE Charris; GM Lobo; MJ Alkaraj. *Free Radic Biol Med*, **2001**, 30, 43.
9. F Herencia; ML Fernandiz; A Udeba; I Guillen; JN Dominguez; JE Charris; GM Lobo; MJ Alkaraj. *FEBS Lett*, **1999**, 453, 129.
10. F Herencia; MP Lopez-garcia; A Ubeda; ML Fernandiz. *Nitic Oxide*, **2002**, 6, 242.
11. DC Noyce; WA Pryor; PA King. *J Am Chem Soc*, **1959**, 81, 5423.
12. ML Go; X Wu; XL Liu. Curr Med Chem, **2005**, 12, 483.
13. J Rojas; M Paya; JN Dominguez; ML Fernandiz. Eur J Pharm Sci, **2003**, 465, 183.