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Synthesis of Some 4'-Amino Chalcones and their Antiinflammatory and Antimicrobial Activity

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A series of some 4'-amino chalcones were synthesized by Claisen-Schmidt condensation of 4-amino acetophenone with various substituted aromatic aldehydes. The synthesized chalcones were characterized by IR, ^{1}H NMR and elemental analyses. When these chalcones (**3a-h**) were evaluated for antiinflammatory, antibacterial and antifungal activities, some of them found to possess significant biological activity when compared to standard drugs.

Key Words: Chalcone, Synthesis, Antiinflammatory, Antimicrobial activity.

INTRODUCTION

Chalcone is a generic term given to compounds bearing the 1,3-diphenylprop-2-en-1-one frame work, which can be functionalized in the propane chain by the presence of olefinic, keto and/or hydroxyl groups (Fig. 1)[1]. The chalcone's bactericidal effect has been related to the ability of the α,β-unsaturated ketone to undergo a conjugated addition to a nucleophillic group like a thiol group in an essential protein. In addition, chalcone derivatives showed activity against dermatophytes only but not against to other types of fungi. Chalcones are readily synthesized by the base catalyzed Claisen-Schmidt condensation of an aldehyde and an appropriate ketone in a polar solvent like ethanol and yields may be variable[2,3], ranging from 5 to 80 %. The chalcones have a diverse range of biological activities, among which antimalarial, antitubercular, cytotoxic, anti-HIV, antiinflammatory, antiplas-modial, immuno-suppressive, antioxidant, analgesic, antiviral and antimicrobial[4-8] properties were widely cited.

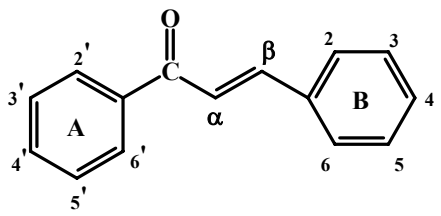
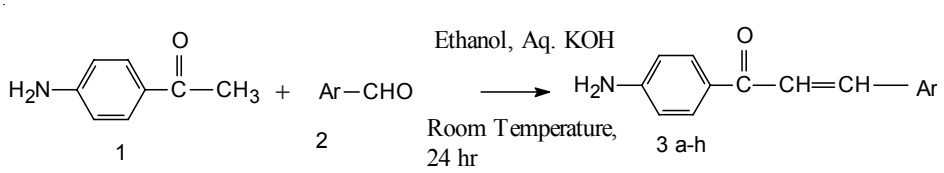
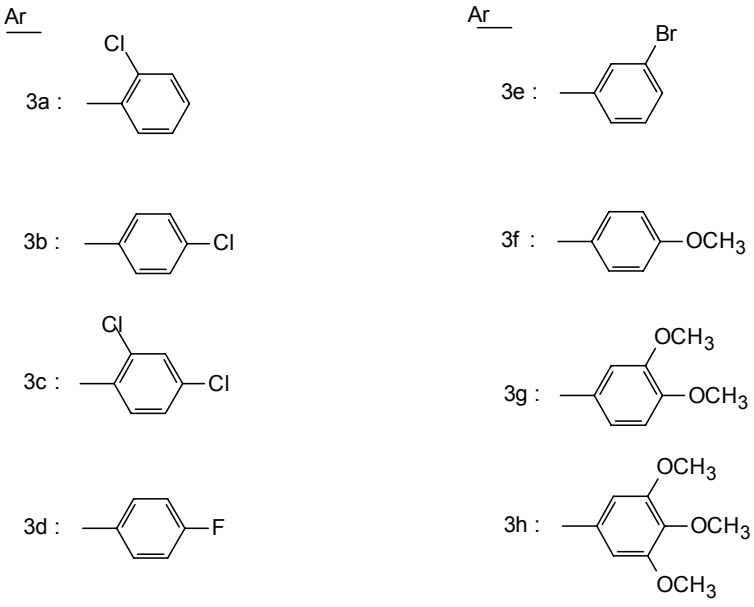


Fig. 1

As shown in **Scheme-I**, 4'-aminochalcones (**3a-h**) were synthesized by a base-catalyzed condensation of appropriately substituted aldehydes and 4-amino aceto-phenone[9]. The structures of various synthesized chalcones were characterized on the basis of elemental analyses, IR and ^{1}HNMR spectral data. The compounds were evaluated for their antiinflammatory activity by carrageenan induced rat paw edema method and antimicrobial activity by agar cup plate method.





Scheme-I

EXPERIMENTAL

Melting points were determined on an open capillary melting point apparatus and are uncorrected. ^{1}H NMR were recorded in $CDCl_3$ on Bruker WM 400 MHz spectrometer with TMS as internal standard. Infrared spectra were recorded (KBr) on a Perkin-Elmer AC-1 spectrophotometer. Microanalyses were performed on Carlo Erba EA-1108 element analyzer and were within the ± 0.4 % of the theoretical values. Reaction completion was identified by TLC using Silica gel-G for TLC (Merck). All the chalcones have been purified by column chromatography performed on Silica gel (100-200 mesh, Merck).

General procedure for the preparation of 1-(4'-amino phenyl)-3-phenyl-2-propen-1-ones (3a-h): Equimolar quantity (0.001 mol) of 4-amino acetophenone and respective aryl aldehyde were mixed and dissolved in minimum amount of alcohol. To this, aqueous potassium hydroxide solution (0.003 mol) was added slowly and mixed occasionally for 24 h, at room temperature and then poured into crushed ice and if necessary acidified with dil. HCl. The solid separated was filtered and dried. It was purified by column chromatography using ethyl acetate and hexane mixture as mobile phase (**Scheme-I**). The physical and spectral data of the chalcones (**3a-h**) are shown in Tables 1 and 2.

TABLE-1
PHYSICAL DATA OF THE PREPARED COMPOUNDS **3a-h**

Compd.	m.p. (ºC)	Yield (%)	m.f. (m.w.)	Elemental analysis %: Found (Calc.d)		
				C	H	N
3a	108	78	$C_{15}H_{12}NOCl$ (257.5)	69.90 (69.97)	4.66 (4.70)	5.43 (5.44)
3b	158	66	$C_{15}H_{12}NOCl$ (257.5)	69.90 (69.97)	4.66 (4.70)	5.43 (5.44)
3c	180	85	$C_{15}H_{11}NOCl_2$ (292)	61.64 (61.70)	3.77 (3.79)	4.79 (4.79)
3d	142	74	$C_{15}H_{12}NOF$ (241)	74.68 (74.76)	4.97 (5.01)	5.80 (5.81)
3e	168	87	$C_{15}H_{12}NOBr$ (302)	59.60 (59.66)	3.97 (4.00)	4.63 (4.64)
3f	108	64	$C_{16}H_{15}NO_2$ (253)	75.88 (75.96)	5.92 (5.97)	5.53 (5.54)
3g	142	72	$C_{17}H_{17}NO_3$ (283)	72.08 (72.15)	6.00 (6.05)	4.94 (4.94)
3h	160	71	$C_{18}H_{19}NO_4$ (313)	69.00 (69.07)	6.07 (6.12)	4.47 (4.47)

Antiinflammatory activity: Spraygue-Dawley rats (M/S Gosh enterprises, Calcutta, West Bengal, India) of either sex weighing between 180-200 g were used in the experiment. 1 % Carrageenan sodium gel was prepared with saline water for producing inflammation and gel of 1 % sodium CMC was prepared with saline water for suspending the test compounds and standard drug.

Rats were divided into ten groups of five animals each. Inflammation was induced by injecting 0.05 mL of 1 % carrageenan subcutaneously into the sub plantar region of the right hind paw and 0.05 mL of saline was injected into the sub plantar region of the left hind paw for all groups. Prior to 1 h, carrageenan injection, the groups **III-X** treated with compounds **3a-h** (10 mg/kg). 1 % Sodium CMC gel (1 mL/kg), was given to group-**I** used as carrageenan treated control and the standard drug aceclofenac (2 mg/kg) was administered to group-**II**. All the doses were administered orally. Antiinflammatory activity was evaluated by measuring carrageenan induced paw oedema[10,11]. The thickness of rat paw was measured before carrageenan injection

TABLE-2
SPECTRAL DATA OF THE PREPARED COMPOUNDS **3a-h**

Compd.	IR (ν_{max} cm^{-1})	^1H NMR ($CDCl_3$), δ ppm
3a	3384, 3332 (N-H), 1647 (C=O), 1607 (CH=CH), 1340 (C-N), 1178 (C-Cl)	4.09 (2H, br S, NH_2), 6.62 (2H, d, *J* = 8.8 Hz, C-3' and 5'-H), 7.26–7.21 (2H, m, C-4 and 5-H), 7.37-7.34 (1H, m, C-3-H), 7.41 (1H, d, *J* = 15.6 Hz, -CO-CH=), 7.67-7.64 (1H, m, C-6-H), 7.85 (2H, d, *J* = 8.4 Hz, C-2' and 6'-H), 8.05 (1H, d, *J* = 15.6 Hz, Ar-CH=) .
3b	3459, 3341 (N-H), 1645 (C=O), 1629 (CH=CH), 1346 (C-N), 1176 (C-Cl)	4.19 (2H, br S, NH_2), 6.72 (2H, d, *J* = 10 Hz, C-3' and 5'-H), 7.22 (1H, d, *J* = 16 Hz, -CO-CH=), 7.38 (2H, d, *J* = 8 Hz, C-3 and 5-H), 7.73 (2H, d, *J* = 8.8 Hz, C-2' and 6'-H), 7.93 (2H, d, *J* = 10 Hz, C-2 and 6-H), 8.02 (1H, d, *J* = 16 Hz, Ar-CH=) .
3c	3436, 3362 (N-H), 1651 (C=O), 1609 (CH=CH), 1343 (C-N), 1180 (C-Cl)	4.20 (2H, br S, NH_2), 6.71 (1H, d, *J* = 15 Hz, -CO-CH=), 7.31 (1H, d, *J* = 8.5 Hz, C-6-H), 7.47 (2H, d, *J* = 10 Hz, C-3' and 5'-H), 7.56 (1H, d, *J* = 8.2 Hz, C-5-H), 7.72-7.68 (1H, m, C-3-H), 7.93 (1H, d, *J* = 16 Hz, Ar-CH=), 8.06 (2H, d, *J* = 8 Hz, C-2' and 6'-H) .
3d	3460, 3340 (N-H), 1628 (C=O), 1603 (CH=CH), 1345 (C-N), 1223 (C-F)	4.20 (2H, br S, NH_2), 6.62 (2H, d, *J* = 8.4 Hz, C-3 and 5-H), 7.03 (2H, d, *J* = 8.8 Hz, C-3' and 5'-H), 7.38 (1H, d, *J* = 15.6 Hz, -CO-CH=), 7.53 (2H, d, *J* = 10.5 Hz, C-2 and 6-H), 7.66 (1H, d, *J* = 15.6 Hz, Ar-CH=), 7.85 (2H, d, *J* = 8.4 Hz, C-2' and 6'-H)
3e	3414, 3326 (N-H), 1652 (C=O), 1626 (CH=CH), 1304 (C-N), 1177 (C-Br)	4.20 (2H, br S, NH_2), 6.71 (1H, d, *J* = 16 Hz, -CO-CH=), 7.27 (2H, d, *J* = 10 Hz, C-3' and 5'-H), 7.57-7.49 (3H, m, C-4, 5 and 6-H), 7.70 (1H, d, *J* = 15 Hz, Ar-CH=), 7.80 (1H, S, C-2-H), 7.95 (2H, d, *J* = 10 Hz, C-2' and 6'-H)
3f	3467, 3329 (N-H), 1631 (C=O), 1598 (CH=CH), 1342 (C-N), 1230, 1025 (C-O-C)	3.85 (3H, S, OCH_3), 4.20 (2H, br S, NH_2), 6.71 (1H, d, *J* = 15.5 Hz, -CO-CH=), 6.93 (2H, d, *J* = 10 Hz, C-3' and 5'-H), 7.43 (2H, d, *J* = 9 Hz, C-3 and 5-H), 7.60 (2H, d, *J* = 8.8 Hz, C-2 and 6-H), 7.76 (1H, d, *J* = 15.5 Hz, Ar-CH=), 7.94 (2H, d, *J* = 10 Hz, C-2' and 6'-H)
3g	3445, 3351 (N-H), 1641 (C=O), 1597 (CH=CH), 1317 (C-N), 1260, 1023 (C-O-C)	3.84 (3H, S, C-3-OCH_3), 3.86 (3H, S, C-4-OCH_3), 4.20 (2H, br S, NH_2), 6.71 (2H, d, *J* = 8 Hz, C-3' and 5'-H), 7.13-6.79 (3H, m, C-2, 5 and 6-H), 7.30 (1H, d, *J* = 15.5 Hz, -CO-CH=), 7.64 (1H, d, *J* = 15.6 Hz, Ar-CH=), 7.84 (2H, d, *J* = 8.4 Hz, C-2' and 6'-H)
3h	3469, 3344 (N-H), 1630 (C=O), 1604 (CH=CH), 1316 (C-N), 1219, 1026 (C-O-C)	3.90 (3H, S, C-4-OCH_3), 3.93 (6H, S, C-3 and 5- OCH_3), 4.20 (2H, br S, NH_2), 6.71 (2H, d, *J* = 10 Hz, C-3' and 5'-H), 6.86 (2H, S, C-2 and 6-H), 7.43 (1H, d, *J* = 16 Hz, -CO-CH=), 7.72 (1H, d, *J* = 15.5 Hz, Ar-CH=), 7.94 (2H, d, *J* = 8 Hz, C-2' and 6'-H)

and after carrageenan injection at time intervals 0.5, 1, 2, 3, 4 and 6 h using Zeitlin's constant loaded lever method[12]. The per cent inhibition of paw edema thickness was calculated[13]. The results and statistical analysis of antiinflammatory activity of aceclofenac and the compounds tested are shown in Table-3 and Fig. 2.

TABLE-3
ANTIINFLAMMATORY ACTIVITY OF CHALCONE DERIVATIVES **3a-h**

Compd.	% inhibition ± SEM at various time intervals					
	0.5 h	1.0 h	2.0 h	3.0 h	4.0 h	6.0 h
Standard	20.26±0.90	23.95±0.97	58.00±1.52	67.93±1.68	97.09±1.97	99.98±2.00
3a	20.68±0.90	25.64±1.00	58.90±1.53	68.11±1.25	95.65±2.00	96.05±2.22
3b	26.29±0.78*	35.67±0.99*	68.57±1.65*	89.97±1.65*	99.38±2.01	99.38±1.99
3c	21.15±0.65	31.56±1.12*	43.23±1.36*	69.93±1.67	92.75±2.32	92.65±2.32
3d	14.23±0.75*	23.07±0.89	45.47±1.45*	77.91±1.33*	88.84±1.88	92.80±2.53
3e	21.56±0.92	26.43±0.99	66.12±1.52*	70.34±1.67	96.94±1.85	96.35±1.84
3f	14.88±0.77*	35.47±0.92*	61.14±1.35	82.45±1.81*	94.10±2.95	97.40±3.25
3g	20.01±0.65*	38.25±1.23*	57.20±1.48	65.24±1.59	90.00±2.32	90.25±2.31
3h	24.78±0.88	31.12±1.02*	51.55±1.43	66.18±1.53	82.56±1.95	94.62±2.35

All values are represented as mean ± SEM (n = 5).
*$p < 0.01$ compared to reference standard Aceclofenac. Student's t-test.

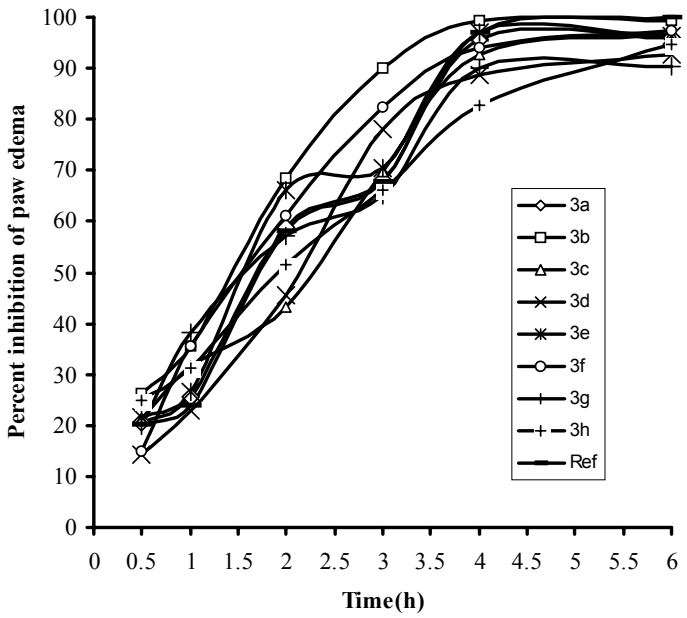


Fig. 2. Antiinflammatory activity of the chalcone derivatives, **3a-h**

Antimicrobial activity: Cup plate method[14,15] using Mueller-Hinton agar medium was employed to study the preliminary antibacterial activity of compounds, **3a-h** against three gram positive bacteria *viz.*, *B. pumilis*, *B. subtilis* and *S. aureus* and two gram negative bacteria *viz.*, *E. coli* and *P. vulgaris*. The agar medium was purchased from HI-Media laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure. Each test compound (5 mg) was dissolved in DMSO (5 mL).

Amikacin and Pencillin-G were employed as reference standards (1000 µg/mL of each) to compare the results. The pH of all the test solutions and control was maintained at 2-3 by using conc. HCl, because the compounds were not diffused through agar medium at pH below 3. All the compounds were tested at a concentration of 0.05 mL (50 µg) and 0.1 mL (100 µg) level and DMSO as a control did not show any inhibition.

Same cup plate method using potato dextrose agar (PDA) medium was employed to study the preliminary antifungal activity of chalcones, **3a-h** against *A. niger, C. albicans* and *R. oryzae*. The PDA medium was purchased from HI-Media laboratories Ltd., Mumbai, India. Preparations of nutrient broth, subculture, base layer medium and PDA medium were done as per the standard procedure. Each test compound (5 mg) was dissolved in DMSO (5 mL). Fluconazole employed as reference standard (1000 µg/mL) to compare the results. The pH of all the test solutions and control was maintained at 2-3 by using conc. HCl, because the compounds were not diffused through agar medium at pH below 3. All the compounds were tested at a concentration of 0.05 mL (50 µg) and 0.1 mL (100 µg) level and DMSO as a control did not show any inhibition.

The cups each of 8 mm diameter were made by scooping out medium with a sterilized cork borer from a petridish which was inoculated with the organisms. The solutions of each test compound, control and reference standard(s) (0.05 and 0.1 mL) were added separately in the cups and petridishes were subsequently incubated at 37 ± 1 ºC for 24 h for antibacterial activity and kept aside at room temperature for 48 h for antifungal activity. Zone of inhibition produced by each compound was measured in mm and the results are presented in Table-4 for antibacterial and in Table-5 for antifungal activity.

TABLE-4
ANTIBACTERIAL ACTIVITY OF CHALCONES **3a-h**

Compd.	Zone of inhibition (in mm) (in mL)									
	B. subtilis		*B. pumilis*		*S. aureus*		*E. coli*		*P. vulgaris*	
	0.05	0.10	0.05	0.10	0.05	0.10	0.05	0.10	0.05	0.10
3a	15	20	15	20	14	20	12	18	15	18
3b	18	24	20	24	18	22	18	25	18	22
3c	16	20	16	20	16	20	15	18	14	19
3d	13	20	14	20	12	20	11	20	12	20
3e	18	24	20	25	18	22	18	24	20	24
3f	14	22	19	24	15	16	12	16	17	24
3g	20	23	21	24	19	23	17	18	19	23
3h	20	24	20	22	18	18	17	17	17	22
Amikacin	28	33	31	32	24	25	25	27	28	31
Pencillin-G	11	11	7	7	15	15	8	8	8	8

TABLE-5
ANTIFUNGAL ACTIVITY OF CHALCONES **3a-h**

Compd.	Zone of inhibition (in mm) (in mL)					
	A. niger		*C. albicans*		*R. oryzae*	
	0.05	0.10	0.05	0.10	0.05	0.10
3a	13	15	15	16	14	15
3b	12	15	12	18	13	18
3c	19	25	21	26	23	25
3d	12	18	14	20	14	19
3e	15	15	14	16	14	17
3f	13	18	14	18	14	20
3g	10	14	12	12	12	14
3h	11	15	13	15	12	16
Fluconazole	24	28	24	28	22	27

RESULTS AND DISCUSSION

From the results, it was noticed that all the chalcone derivatives tested showed considerable antiinflammatory activity. In addition, it has been found that **3b** showed maximum activity when compared to aceclofenac and this may be due to the presence of chlorine at 4-position on aromatic ring-B of chalcone. Moreover, it was also observed that the compounds **3d** and **3f** having fluorine and methoxyl at *para* position of the aromatic ring respectively exhibited better activity.

Compounds **3a-h** showed significant antibacterial activity at both 0.05 mL (50 µg) and 0.1 mL (100 µg) concentration level when compared with standard amikacin and pencillin-G. In particular compounds **3b**, **3e**, **3g** and **3h** possessed maximum activity which may be due to the presence of chlorine at C-4, bromine at C-3, methoxyl at C-3 and 4 and also methoxyl at C-3, 4 and 5, respectively on aromatic ring-B.

The results of antifungal activity revealed that the compounds, **3a-h** exhibited moderate to considerable activity when compared with reference standard, fluconazole at both 0.05 mL (50 µg) and 0.1 mL (100 µg) concentration level. Compounds **3c**, **3e** and **3f** carrying chlorine at 2- and 4- position (**3c**), bromine at 3- position (**3e**) and methoxyl at 4-position (**3f**) on the aromatic ring-B showed remarkable activity.

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