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SYNTHESIS AND STUDIES OF ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITY OF SOME NEW 4'-AMINO CHALCONES

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ABSTRACT

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

Key words : Chalcone, Synthesis, Anti-inflammatory, 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]. Their 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 other types of fungi. Chalcones are readily synthesized by the base catalysed Claisen-Schmidt condensation of an aldehyde and an appropriate ketone in a polar solvent like ethanol and yields may be variable, ranging from 5% to 80%[2, 3]. The chalcones have a diverse range of biological activities, among which antimalarial, antitubercular, cytotoxic, anti-HIV, anti-inflammatory, antiplasmodial, immunosuppressive, antioxidant, analgesic, antiviral and antimicrobial[4-8] properties were widely cited.

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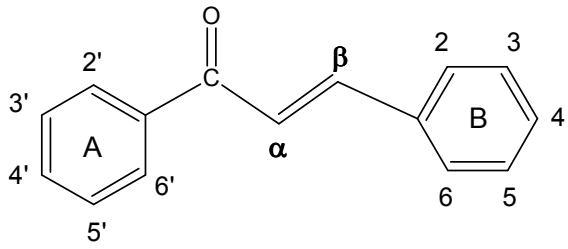


Fig. 1

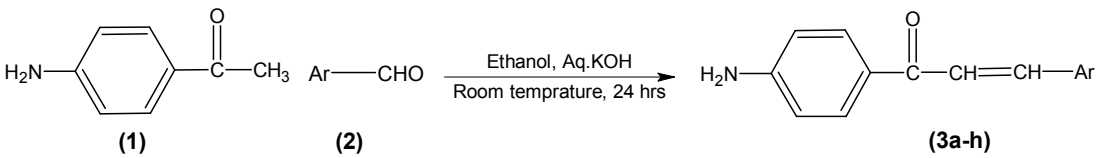
As shown in **Scheme 1**, 4'-aminochalcones **(3a-h)** were synthesized by a base-catalyzed condensation of appropriately substituted aldehydes and 4-amino acetophenone[9]. The structures of various synthesized chalcones were characterized on the basis of elemental analyses, IR and ^{1}H NMR spectral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

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 columns (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 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 hrs, 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 1)**. The physical and spectral data of the chalcones **(3a-h)** are shown in Table 1 and Table 2.



(1) **(2)** **(3a-h)**

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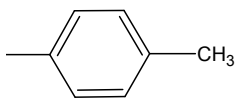
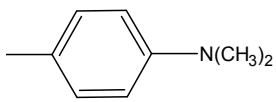
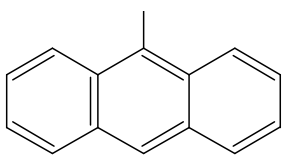
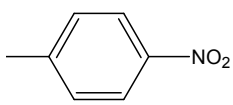
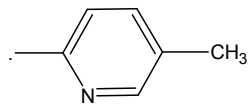
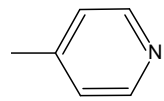
(3a) 

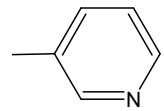
(3b) 

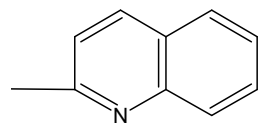
(3c) 

(3d) 

(3e) 

(3f) 

(3g) 

(3h) 

Scheme 1

Table 1. Physical data of the prepared compounds (3a-h)

Compound	Melting point (^{0}C)	% Yield	Molecular formula (Mol. wt.)	% Analysis, Found (Calc.)		
				C	H	N
(3a)	122	62	$C_{16}H_{15}NO$ (237)	81.01 (81.08)	6.32 (6.32)	5.90 (5.91)
(3b)	172	65	$C_{17}H_{18}N_2O$ (266)	76.69 (76.76)	6.76 (6.82)	10.52 (10.53)

Cont…

Compound	Melting point (^{0}C)	% Yield	Molecular formula (Mol. wt.)	% Analysis, Found (Calc.)		
				C	H	N
(3c)	170	62	$C_{23}H_{17}NO$ (323)	85.44 (85.52)	5.26 (5.30)	4.33 (4.33)
(3d)	182	60	$C_{15}H_{12}N_2O_3$ (268)	67.16 (67.22)	4.47 (4.51)	10.44 (10.45)
(3e)	142	62	$C_{14}H_{12}N_2O$ (224)	75.00 (75.06)	5.35 (5.39)	12.50 (12.50)
(3f)	170	55	$C_{14}H_{12}N_2O$ (224)	75.00 (75.06)	5.35 (5.39)	12.50 (12.50)
(3g)	160	60	$C_{14}H_{12}N_2O$ (224)	75.00 (75.06)	5.35 (5.39)	12.50 (12.50)
(3h)	122	58	$C_{18}H_{14}N_2O$ (274)	78.83 (78.90)	5.10 (5.15)	10.21 (10.22)

Table 2 . Spectral data of the prepared compounds (3a-h)

Compd.	IR (υ_{max}, cm^{-1})	^{1}H NMR ($CDCl_3$), δ ppm
(3a)	3471, 3348 (N-H), 1628 (C=O), 1602 (CH=CH),1337 (C-N)	2.42 (3H, S, C-4-CH_3), 4.20 (2H, br S, NH_2), 6.81 (2H, d, J = 8 Hz, C-3' and 5'-H), 7.05 (1H, d, J = 15.4 Hz, -CO-CH=), 7.23 (2H, d, J = 7.5 Hz, C-2 and 6-H), 7.53 (2H, d, J = 8 Hz, C-3 and 5-H), 7.73 (1H, d, J = 16 Hz, Ar-CH=), 8.03 (2H, d, J = 10 Hz, C-2' and 6'-H)
(3b)	3474, 3432 (N-H), 1620 (C=O), 1597 (CH=CH), 1346 (C-N), 1303 (C-N-C)	3.05 [6H, S, Ar-N(CH_3)$_2$], 4.19 (2H, br S, NH_2), 6.70 (2H, d, J = 10 Hz, C-3' and 5'-H), 6.93-6.78 (2H, m, C-3 and 5-H), 7.39 (1H, d, J = 15 Hz, -CO-CH=), 7.57 (2H, d, J = 10 Hz, C-2 and 6-H), 7.76 (1H, d, J = 15 Hz, Ar-CH=), 7.94 (2H, d, J = 8 Hz, C-2' and 6'-H)

Compd.	IR (υ_{max}, cm^{-1})	^1H NMR ($CDCl_3$), δ ppm
(3c)	3469, 3332 (N-H), 1650 (C=O), 1633 (CH=CH),1356 (C-N)	4.20 (2H, br S, NH_2), 7.22 (1H, d, J = 16 Hz, -CO-CH=), 7.53-7.45 (4H, m, Ar-H-anthracenyl),7.57 (2H, d, J = 8 Hz, C-3' and 5'-H), 8.03 (1H, d, J = 15.5 Hz, Ar-CH=), 8.15-8.07 (2H, m,Ar-H-anthracenyl), 8.30 (2H, d, J = 8 Hz, C-2' and 6'-H), 8.88-8.45 (3H, m, Ar-H-anthracenyl)
(3d)	3484, 3389 (N-H), 1636 (C=O), 1610 (CH=CH),1341 (C-N), 1506,1317[$(N=O)_2$, Ar-NO_2],874 (C-N, Ar-NO_2)	4.20 (2H, br S, NH_2), 6.68 (2H, d, J = 10 Hz, C-3' and 5'-H), 6.72 (1H, d, J = 16 Hz, -CO-CH=),7.66 (2H, d, J = 10 Hz, C-2 and 6-H), 7.80 (1H, d, J = 16 Hz, Ar-CH=), 7.95 (2H, d, J = 10 Hz, C-2' and 6'-H), 8.28 (2H, d, J = 10 Hz, C-3 and 5-H)
(3e)	3399 (N-H), 1646 (C=O),1585 (CH=CH), 1432 (C=N), 1336 (C-N)	4.12 (2H, br S, NH_2), 6.62 (2H, d, J = 8.8 Hz, C-3' and 5'-H), 7.22-7.19 (1H, m, C-4-H),7.38 (1H, d, J = 15 Hz, -CO-CH=), 7.67-7.63 (1H, m, C-5-H), 7.68 (1H, d, J = 8 Hz, C-6-H), 7.93 (2H, d, J = 10 Hz, C-2' and 6'-H), 8.06 (1H, d, J = 8.8 Hz, C-3-H), 8.60 (1H, d, J = 15.5 Hz, Ar-CH=)
(3f)	3439 (N-H), 1643 (C=O),1593 (CH=CH), 1414 (C=N), 1336 (C-N)	4.16 (2H, br S, NH_2), 6.63 (2H, d, J = 8.4 Hz, C-3' and 5'-H), 7.38 (2H, d, J = 18 Hz, -CO-CH= and C-3-H), 7.60 (2H, d, J = 8 Hz, C-2 and 6-H), 7.85 (2H, d, J = 8.4 Hz, C-2' and 6'-H), 8.60 (2H, d, J = 18.5 Hz, Ar-CH= and C-5-H)
(3g)	3437 (N-H), 1636 (C=O), 1595 (CH=CH), 1422 (C=N), 1342 (C-N)	4.13 (2H, br S, NH_2), 6.63 (2H, d, J = 8.8 Hz, C-3' and 5'-H), 7.26 (1H, d, J = 8 Hz, C-4-H),7.52 (1H, d, J = 15.6 Hz, -CO-CH=), 7.68 (1H, d, J = 16 Hz, Ar-CH=), 7.86 (2H, d, J = 8.4 Hz, C-2' and 6'-H), 8.54-8.52 (1H, m, C-5-H), 8.78 (2H, d, J = 9 Hz, C-2 and 6-H)

Compd.	IR (υ_{max}, cm^{-1})	^1H NMR ($CDCl_3$), δ ppm
(3h)	3470, 3357 (N-H), 1638 (C=O), 1605 (CH=CH),1524 (C=N),1357 (C-N)	4.15 (2H, br S, NH_2), 6.73 (2H, d, J = 8 Hz, C-3' and 5'-H), 7.70 (1H, d, J = 15 Hz, -CO-CH=),7.93 (1H, d, J = 16 Hz, Ar-CH=), 8.06 (1H, d, J = 12 Hz, C-2' and 6'-H),8.40-7.61 (6H, m, Ar-H-quinolinyl)

Anti-inflammatory 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. One hour prior to 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. Anti-inflammatory activity was evaluated by measuring carrageenan induced paw oedema[10, 11]. The thickness of rat paw was measured before carrageenan injection 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 percentage increase of paw edema thickness was calculated[13]. The results and statistical analysis of anti-inflammatory activity of aceclofenac and the compounds tested are shown in Table 3 and Fig. 2**.**

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 dimethyl sulfoxide (5 mL). Amikacin sulphate and pencillin-G were

Table 3. Anti-inflammatory activity of chalcone derivatives (3a-h)

Compd.	% Inhibition ± SEM at various time intervals					
	0.5 hr	1.0 hr	2.0 hr	3.0 hr	4.0 hr	6.0 hr
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)	25.27 ± 1.00	30.49 ± 1.10*	26.68 ± 1.03	69.63 ± 1.66	92.70 ± 2.02	94.67 ± 2.12
(3b)	17.44 ± 0.75	19.55 ± 0.89	36.50 ± 1.15*	62.85 ± 1.75	81.28 ± 1.80	87.35 ± 2.32
(3c)	21.70 ± 0.93	25.56 ± 1.01	36.96 ± 1.35*	78.94 ± 1.77*	87.14 ± 2.05	97.40 ± 2.36
(3d)	11.90 ± 0.69*	12.21 ± 0.69*	42.89 ± 1.85*	62.55 ± 2.03	79.65 ± 2.32*	97.35 ± 2.98
(3e)	26.09 ± 1.02	27.04 ± 1.05	37.85 ± 1.36*	87.41 ± 2.06	89.80 ± 2.31	85.96 ± 2.85
(3f)	28.42 ± 1.23*	28.79 ± 1.32	36.84 ± 1.52*	67.31 ± 1.98	85.21 ± 2.05*	97.26 ± 2.56
(3g)	14.72 ± 0.75	18.75 ± 0.85	36.46 ± 1.32*	67.07 ± 1.92	92.50 ± 2.35	87.26 ± 2.65
(3h)	29.21 ± 1.35*	30.26 ± 1.36	46.92 ± 1.45*	80.27 ± 1.96*	98.47 ± 2.72	98.61 ± 2.84

All values are represented as mean ± SEM (n = 5).

*P < 0.01 compared to reference standard Aceclofenac. student's t-test.

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.

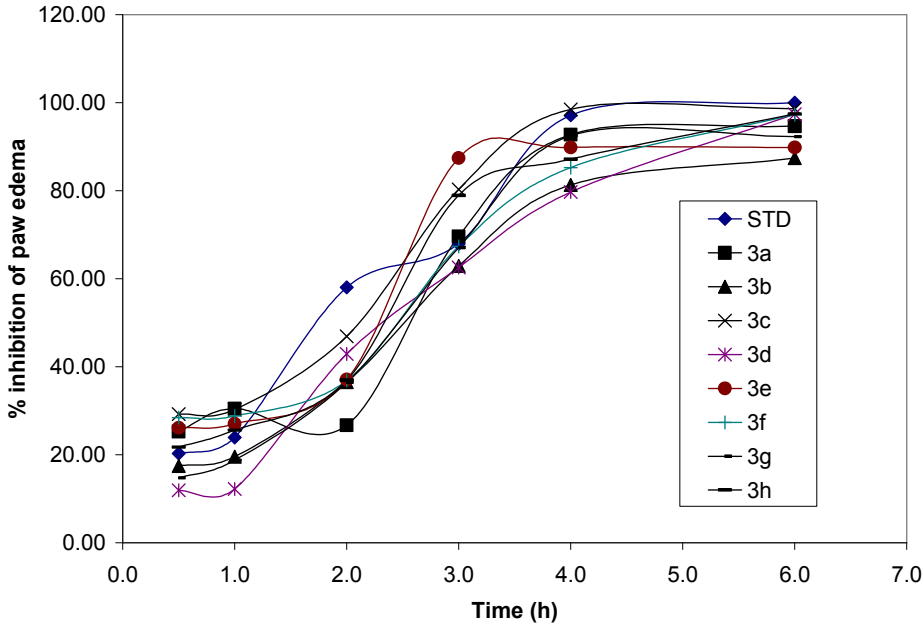


Fig. 2 : Anti-inflammatory activity of chalcone derivatives (3a-h)

Same cup plate method using PDA (potato dextrose agar) medium was employed to study the preliminary antifungal activity of chalcones **(3a-h)** against *A. niger, C. albican*, and *R.oriza*. 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 dimethyl sulfoxide (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 mL and 0.1 mL) were added separately in the cups and petridishes and were subsequently incubated at 37ºC ± 1ºC for 24 hrs for antibacterial activity and kept aside at room temperature for 48 hrs 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)									
	B. subtilis		***B. pumilis***		***S. aureus***		***E. coli***		***P. vulgaris***	
	0.05 mL	**0.10 mL**	**0.05 mL**	**0.10 mL**	**0.05 mL**	**0.10 mL**	**0.05 mL**	**0.10 mL**	**0.05 mL**	**0.10 mL**
(3a)	20	28	21	28	22	29	20	26	20	25
(3b)	21	28	22	29	22	27	21	27	20	27
(3c)	18	20	18	21	17	22	18	20	16	20
(3d)	17	23	16	20	16	21	16	18	16	19
(3e)	18	23	18	20	18	21	17	18	17	20
(3f)	17	21	16	22	19	20	16	19	15	20
(3g)	16	21	16	22	16	21	16	20	18	21
(3h)	19	22	16	23	15	20	17	19	17	20
Amikacin	32	35	30	34	25	30	24	30	31	31
Pencillin-G	10	11	10	10	10	10	10	10	10	10

Table 5. Antifungal activity of chalcones (3a-h)

Compd.	Zone of inhibition (in mm)					
	A. niger		***C. albican***		***R. oriza***	
	0.05 mL	**0.10 mL**	**0.05 mL**	**0.10 mL**	**0.05 mL**	**0.10 mL**
(3a)	19	24	19	23	20	25
(3b)	22	26	23	26	20	24
(3c)	12	15	12	16	11	14

Cont…

Compd.	Zone of inhibition (in mm)					
	A. niger		***C. albican***		***R. oriza***	
	0.05 mL	**0.10 mL**	**0.05 mL**	**0.10 mL**	**0.05 mL**	**0.10 mL**
(3d)	13	17	11	16	12	17
(3e)	14	15	13	17	11	16
(3f)	13	18	14	17	12	18
(3g)	13	16	13	18	13	18
(3h)	13	17	12	16	12	18
Flucon -azole	24	28	24	28	22	27

RESULTS AND DISCUSSION

The results of anti-inflammatory activity revealed that the compounds **(3a-h)** exhibited moderate to considerable activity when compared with reference standard aceclofenac. In addition, it was found that **(3d)** showed maximum activity and this may be due to the presence of nitro group at 4-position on aromatic ring-B of chalcone. Moreover, it was also observed that the compounds **(3f)** and **(3h),** carrying 4-pyridinyl and 2-quinolinyl as ring-B of chalcone, respectively, showed remarkable activity.

Compounds **(3a-h)** showed significant antibacterial activity at both 0.05 mL (50 μg) and 0.1 mL (100 μg) concentration levels when compared with standard drugs amikacin and pencillin-G. In particular compounds **(3a)** and **(3b)** possessed maximum activity. From the obtained results, it was clear that all the chalcone derivatives tested showed considerable antifungal activity, when compared with reference standard, fluconazole at both 0.05 mL (50 μg) and 0.1 mL (100 μg) concentration level. Compounds **(3a)** and **(3b)** showed maximum antifungal activity. From the above results, it is interesting to note that the diaryl chalcones, which are having electron releasing substituents like methyl and dimethyl amine at C-4 position of aromatic ring-B showed moderate to considerable antibacterial and antifungal activities, when compared to that of heteroaryl chalcones.

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