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ANTI-INFLAMMATORY ACTIVITY OF SOME NEWLY SYNTHESIZED CHALCONES

Jahirul Islam Talukdar[1,]*, Monica Kachroo[1] and Rema Razdan[2]

[1]Department of Pharmaceutical Chemistry, Al-Ameen College of Pharmacy, Hosur road, Bangalore-560027, Karnataka, India.
[2]Department of Pharmacology, Al-Ameen College of Pharmacy, Hosur road, Bangalore-560027, Karnataka, India.

***Corresponding author e-mail:** jahirul_1987@yahoo.com

ABSTRACT

A new series of chalcones have been synthesized by Claisen-Schmidt condensation of appropriate acetophenones like *N-(4-acetyl-phenyl)-4-methoxy-benzamide* [which is synthesized by reacting 4-methoxy-benzoyl chloride with 4-amino acetophenone] and *N-(4-acetyl-phenyl)-4-chloro-benzamide*, [which is synthesized by reacting 4-chloro-benzoyl chloride with 4-amino acetophenone] with various aldehydes in ethanolic KOH solution. The synthesized compounds were characterized using melting point, TLC, UV, IR, ^{1}HNMR, ^{13}C-NMR and CHN analysis. The compounds were evaluated for their anti-inflammatory activity by bovin serum albumin assay method (*in vitro*) and carrageenan induced rat paw oedema method (*in vivo*). Among the compounds synthesized, 2, 4-dimethoxy phenyl and 4-ethoxy phenyl derivative exhibited significant anti-inflammatory activity.

Key words: Chalcones, Claisen-Schmidt, anti-inflammatory activity.

INTRODUCTION

Chalcones are α, β- unsaturated ketones consisting of two aromatic rings having diverse array of substituents. Rings are interconnected by a highly electrophilic three carbon α, β- unsaturated carbonyl system that assumes linear or nearly planer structure. They contain the ketoethylenic group[1]. Chalcones are the important constituents of natural sources. It was first isolated from Chinese liquorice. The 1, 3-diaryl-1-ones skeletal system was recognized as the main pharmacophore for chalcones. From plants , stable chalcone moiety can not be isolated because the presence of enzyme chalcone synthetase, which immediately converts chalcone into flavanone[2]. Several strategies for the synthesis of α, β-unsaturated carbonyl system based on formation of carbon- carbon bond have been reported and among them Claisen-Schmidt condensation still occupy prominent position[3]. According to literature survey, it was noted that many of the chalcone derivatives has been synthesized and found to exhibit anti-inflammatory[4], antitubercular[5], antioxidant[6], antibacterial activity[7], hence an attempt has been made in the present study to synthesize some new chalcone derivatives and evaluate the compounds for anti-inflammatory activity.

MATERIALS AND METHODS

General: The chemicals and reagents used in the project were of AR and LR grade, procured from Aldrich, Hi-media, BDH chemicals, Finer and Merck. Melting points were determined in open capillary tubes on an electrical apparatus and are uncorrected, the IR spectra were recorded using KBr pellets in range of 4000-400 cm^{-1} on a Fourier Transform IR Spectrometer (Shimadzu 8700) and the frequencies are recorded in wave numbers. ^{1}H-NMR (400 MHz) spectra were recorded in $CDCl_3$-d_6 in Amx-400 liquid state PMR spectrometer (Astra Zeneca , Bangalore). Chemical shifts (δ) are reported

in parts per million downfield from internal reference tetramethylsilane (TMS).

Experimental

Synthesis of 4-Substituted benzoyl chloride

4-substituted benzoic acid, thionyl chloride and dry benzene were taken in a 250 ml round bottom flask. Refluxed the reaction mixture for 3 hours. Distilled of the excess solvent and collected the product.

Synthesis of N-(4-acetyl-phenyl)-benzamide

Dichloromethane and *p*-amino acetophenone were taken in a conical flask and stirred continuously. While stirring , added 4-substituted benzoyl chloride dropwise. Rinsed the reaction flask with a few drops of dichloromethane and added this rinse to the conical flask. Stirring was continue for 1-3 hrs. Completion of the reaction was monitored by TLC. Added cold water, filtered the precipitate, collected the solid product and recrystallized using ethanol.

Synthesis of chalcones 6 (a-e) and 7 (a-e)

A mixture of N-(4-acetyl-phenyl)-benzamide and substituted aldehyde was stirred in ethanol and an aqueous solution of KOH was added to it. The stirring was continued for 5 hrs and the mixture was kept overnight at room temperature and it was then poured into crushed ice and acidified with HCl. The solid separated was filtered and recrystallized from ethanol.

N-{4-[3-(2,3-dichloro-phenyl)- acryloyl]-phenyl}-4-methoxy benzamide (6a)

Light yellow solid, 89% yield; FTIR (KBr) υ (cm^{-1}): 1659 (C = O str of α ,β unsaturated ketone) ;1602 (C = O str of amide); 3411 (N – H str).

N-{4-[3-(2,4-dimethoxy-phenyl)- acryloyl]-phenyl}-4-methoxy benzamide (6b)

Yellow solid, 88.54% yield; FTIR (KBr) υ (cm^{-1}): 1645 (C = O str of α ,β unsaturated ketone) ;1591 (C = O str of amide); 3319 (N – H str); 1573 (Ali C = C str); 1213 (C – O – C str of methoxy). ^{1}H NMR ($CDCl_3$) : δ 3.88 (s, 9H, -OCH_3), 8.03 (s, 1H, -N-H), 7.01-7.98 (m, 11H, Ar-H), 6.96 (d, 1H , -COCH=), 8.10 (d, 1H, -Ar-CH=); MS *m/z* : [M^+ + 1]: 418 ; CHN (%): Calculated, C = 71.94, H=5.51 , N= 3.35; Found, C= 71.82, H=5.63, N= 3.45.

N-{4-[3-(2-hydroxy phenyl)- acryloyl]-phenyl}-4-methoxy benzamide (6c)

Brown solid, 48% yield; FTIR (KBr) υ (cm^{-1}): 1650 (C = O str of α ,β unsaturated ketone) ;1598 (C = O str of amide); 3089 (N – H str); 3288 (O-H str); 1519 (Ali C=C).

4-Methoxy-N-{4-[3-(3-nitro-phenyl) - acryloyl]-phenyl}- benzamide (6d)

Light brown solid, 51% yield; FTIR (KBr) υ (cm^{-1}): 1660 (C = O str of α ,β unsaturated ketone) ;1604 (C = O str of amide); 3353 (N – H str); 3288; 1523& 1315 (N-O str of NO_2).

N-{4-[3-(4-ethoxy -phenyl)- acryloyl]-phenyl}-4-methoxy benzamide (6e)

Buff color solid, 77% yield; FTIR (KBr) υ (cm^{-1}): 1662 (C = O str of α ,β unsaturated ketone) ;1598 (C = O str of amide); 3404 (N – H str); 1504 (Ali C = C str); ^{1}H NMR ($CDCl_3$) : δ 3.87 (s, 3H, -OCH_3), 8.03 (s, 1H, N-H), 7.19-7.89 (m, 12H, Ar-H), 7.0 (d, 1H , -COCH=), 7.96 (d, 1H, -Ar-CH=), 3.95-4.10 (q, 2H, CH_2 of ethoxy), 1.40-1.47 (t, 3H, CH_3 of ethoxy); ^{13}C NMR ($CDCl_3$) δ (ppm): 189.37 (C = O), 165.55, 163.26, 161.49, 144.69, 142.60, 134.70, 130.46, 130.16, 130.15, 129.37, 128.11, 127.25, 120.12, 119.74, 115.41, 114.52, 64.06, 55.78, 14.98.

4-Chloro-N-{4-[3-(2,3-dichloro- phenyl)- acryloyl]-phenyl}-benzamide (7a)

Light green solid, 92.35% yield; FTIR (KBr) υ (cm^{-1}): 1679 (C = O str of α ,β unsaturated ketone) ;1658 (C = O str of amide); 3355 (N – H str); 1593 (Ali C=C).

4 -Chloro- N- {4 - [3- (2, 4-dimethoxy -phenyl) - acryloyl] -phenyl}-benzamide (7b)

Pale yellow solid, 84.4% yield; FTIR (KBr) υ (cm^{-1}): 1652 (C = O str of α ,β unsaturated ketone) ;1593 (C = O str of amide); 3330 (N – H str); 1517 (Ali C = C str); 1265 (C – O – C str of methoxy). ^{1}H NMR ($CDCl_3$) : δ 3.86 (s, 6H, -OCH_3), 8.01 (s, 1H, -N-H), 7.26-7.86 (m, 11H, Ar-H), 6.48 (d, 1H , -COCH=), 8.12 (d, 1H, -Ar-CH=); MS *m/z* : [M^+ + 2]: 423 ; CHN (%): Calculated, C = 68.4, H=4.75 , N= 3.32; Found, C= 68.21, H=4.71, N= 3.27.

4-Chloro-N-{4-[3-(2,hydroxy-phenyl)- acryloyl]-phenyl}-benzamide (7c)

Buff color solid, 59% yield; FTIR (KBr) υ (cm^{-1}): 1676 (C = O str of α ,β unsaturated ketone) ;1607 (C = O str of amide); 3280 (N – H str); 1560 (Ali C=C), 3310 (O – H str).

4-Chloro-N-{4-[3-(3-nitro-phenyl)-acryloyl]-phenyl}-benzamide (7d)

Brown solid, 74% yield; FTIR (KBr) υ (cm^{-1}): 1660 (C = O str of α ,β unsaturated ketone) ;1593 (C = O str of amide); 3353 (N – H str); 1525 (Ali C=C), 1483 & 1350 (N – O str of NO_2).

4-Chloro-N-{4-[3-(4-ethoxy-phenyl)- acryloyl]-phenyl}-benzamide (7e)

Light green solid, 82.9% yield; FTIR (KBr) υ (cm^{-1}): 1672 (C = O str of α ,β unsaturated ketone) ;1612 (C = O str of amide); 3379 (N – H str);1406 (Ali C=C).

BIOLOGICAL ACTIVITY

In vitro anti-inflammatory activity

Bovine serum albumin assay seeks to eliminate the use of live specimens as far as possible in the drug developmental process. When BSA is heated, it undergoes denaturation and expresses antigens associated with type III hypersensitive reaction and which are related to diseases such as serum sickness, glomerulonephritis and rheumatoid arthritis. Thus the assay applied for the discovery of those drugs which can stabilize the protein from denaturation process. A solution of 0.2% w/v of BSA was prepared in tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid. Stock solutions of 10000 µg/mL of all test samples were prepared by using methanol as a solvent. From these stock solutions two different concentrations of 100µg/mL and 200 µg/mL were prepared by using methanol as a solvent. 100µl of each test sample was transferred to volumetric flask (10 mL) using 1mL micropipette. 5mL of 0.2% BSA was added to all the above flasks. The control consists of 5mL 0.2%w/v BSA solution with 0.1mL methanol. The 0.1mL standard consist 100µg/mL of diclofenac sodium in methanol with 5mL 0.2%w/v BSA solution. The volumetric flasks were heated at 72^0C for five minutes and then cooled for 10 min. The absorbance of these solutions were determined by using spectrophotometer at a wavelength of 660 nm. The % inhibition of precipitation (denaturation of the protein) was determined on a % basis relative to the control using the following formula.

$$\% \text{ Inhibition} = \frac{[\ AC - AT\ ]}{[\ AC]} \times 100$$

Where AC is the absorbance of control,
AT is the absorbance of test.

Determination of lipophilicity

Since lipophilicity is a significant physico chemical property determining distribution, bioavailability, metabolic activity and elimination, a computational study for prediction of ADME properties of the molecule was performed by determination of lipophilicity, TPSA, and simple molecular descriptors used by Lipinski in formulating his "rule of five" calculations by using www.molinspiration.com.

Table 2 represents the calculated clogP, R_M, SMV, TPSA and other Lipinski parameters of the synthesized compounds. It was interesting to observe that the clogP value of the compounds **6b** and **6d** were 4.39 and 4.69 respectively while all other compounds were found to have more than 5.0. Since the drugs are highly lipophilic we have selected the intraperitoneal route for administration of drugs before carrageenan administration in *in-vivo* anti-inflammatory activity study. The R_M values could be used as a successful relative measure of the overall lipophilic/ hydrophilic balance of the molecules and therefore it was tried to correlate the R_M values with the theoretically calculated clog P values.

In vivo anti-inflammatory activity

In vivo anti-inflammatory activity of the synthesized compounds were carried out by carrageenan induced rat paw oedema method (approved by Institutional Animal Ethics Committee bearing approval No. AACP / IAEC / Nov-2011 / 08).

Carrageenan induced rat paw oedema is a standard and most commonly used technique to screen the *in vivo* anti-inflammatory activity. It is expected that after tissue injury an animal will display spontaneous pain behaviour.This peripheral hypersensitivity or pain perception can be explained on the basis of local release of various inflammatory mediators i.e. bradykinin, prostaglandins or cytokines which can activate and sensitize the peripheral nerve endings. This phenomena can be evaluated by a simple laboratory technique i.e. carrageenan induced paw edema. In the present study the anti-inflammatory activity of some of the synthesized compounds were evaluated in comparison with indomethacin, which is used as a standard. Wistar rats in a number of 40, weighing between 260-300 gm were divided into 8 groups, each containing 5 animals.

The initial paw volume of each rat was noted by mercury displacement method using plethysmograph. Animals in the group-1 were administered vehicle (mixture of 2.5%DMSO + 2.5% tween 80), i.p, group-2 received indomethacin at a dose of 0.01 mmol/kg i.p. Groups 3-8 received the test samples at a dose of 0.01 mmol/kg i.p. After the drug treatment, 1% w/v carrageenan solution (0.1 ml/paw) was injected subcutaneously into the plantar surface of the right hind paw of the rat. The paw volume of control, standard & test groups were measured with the help of plethysmograph during the time interval of 1h, 2h and 3h after carrageenan administration[14].

$\Delta V = Vt - Vo$

Where,

ΔV is the change in paw volume

Vt is the paw volume at 1, 2 and 3h.

Vo is the paw volume at 0h.

Statistical analysis

All data were expressed as mean ± SEM and one-way ANOVA, Turkey's test was applied to determine the significance of the difference between the control groups and rat treated with the test compounds (using graph pad prism software).

RESULTS AND DISCUSSION

The new chalcone derivatives were synthesized by Claisen-Schmidt condensation reaction. The synthesis of chalcone derivatives was performed following the steps shown in **scheme**. In order to obtain the chalcones, the corresponding N-(4-acetyl-phenyl)-benzamide **5a** and **5b** were initially synthesized by reacting respective benzoyl chloride **3a** and **3b** (prepared by condensation of the respective benzoic acid **1a** and **1b** with thionyl chloride **2**) and *p*-amino acetophenone **4**. Compounds **5a** and **5b** were characterized by using IR spectra. Subsequently, the Claisen-Schmidt condensation of the obtained acetophenones **5a** and **5b** with various aldehydes **(a-e)** afforded the corresponding chalcones **6 (a-e)** and **7 (a-e)**.

The formation of the product was monitored by TLC and the structure of the synthesized compounds was confirmed by IR, ^{1}H NMR, ^{13}C NMR, LCMS and elemental analysis. In elemental analysis, the percentage of nitrogen, hydrogen and carbon was found to be experimentally equivalent to the calculated values. LCMS of the synthesized chalcones gave peak for the respective molecular mass.

The ^{1}H NMR of compound **6b** showed a singlet at δ 3.88 for methoxy protons on the phenyl ring and showed a signal at δ 8.03 for amide proton. The signal which appeared in the region at δ 7.01- 7.98 as multiplets was due to the aromatic protons present in the molecules. This data confirmed the formation of **6b**. Similarly compound **6e** showed a singlet at δ 3.87 for methoxy proton, singlet at δ 8.03 for amide proton, multiplets in the region at δ 7.19- 7.89 due to aromatic protons, CH_2 of ethoxy showed quartet at δ 3.95-4.10 and CH_3 ethoxy showed triplet at δ 1.40-1.47. Compound **6e** was further supported by recording ^{13}C NMR spectrum and the signals appeared in the spectrum account for all the C-atoms present in a molecule of **6e.** LC mass spectrum of the compound **6b** showed molecular ion peaks at m/z 418 [M^{+} +1]. Similarly, all other chalcones were characterized and the data are given in the experimental section.

Anti-inflammatory activity: *In vitro* anti-inflammatory activity of the synthesized compounds was carried out by bovin serum albumin assay method. **Figure 1** shows the *in vitro* anti-inflammatory activity of the synthesized compounds compared to the reference drug diclofenac. *In vivo* anti-inflammatory activities of the synthesized compounds were carried out by carrageenan induced rat paw oedema method. **Figure 2** shows the *in vivo* anti-inflammatory activity of the compounds compared to the reference standard drug indomethacin.

CONCLUSION

The main focus of this research work was to synthesize, purify, characterize and evaluate the anti-inflammatory activity of newly synthesized chalcone derivatives. The yield of the synthesized compound was found to be in the range from 48 to 92%. Structure of synthesized compounds was confirmed and characterized with the help of analytical data such as FTIR, ^{1}HNMR, ^{13}C-NMR, Mass spectroscopy and CHN analysis. It was observed from the results obtained that most of the tested compounds showed some anti-inflammatory activity. These results give an idea about the possible importance of the chalcone moiety and also different substituents on the chalcone derivatives. The activity is enhanced due to the presence of methoxy and ethoxy substitution.

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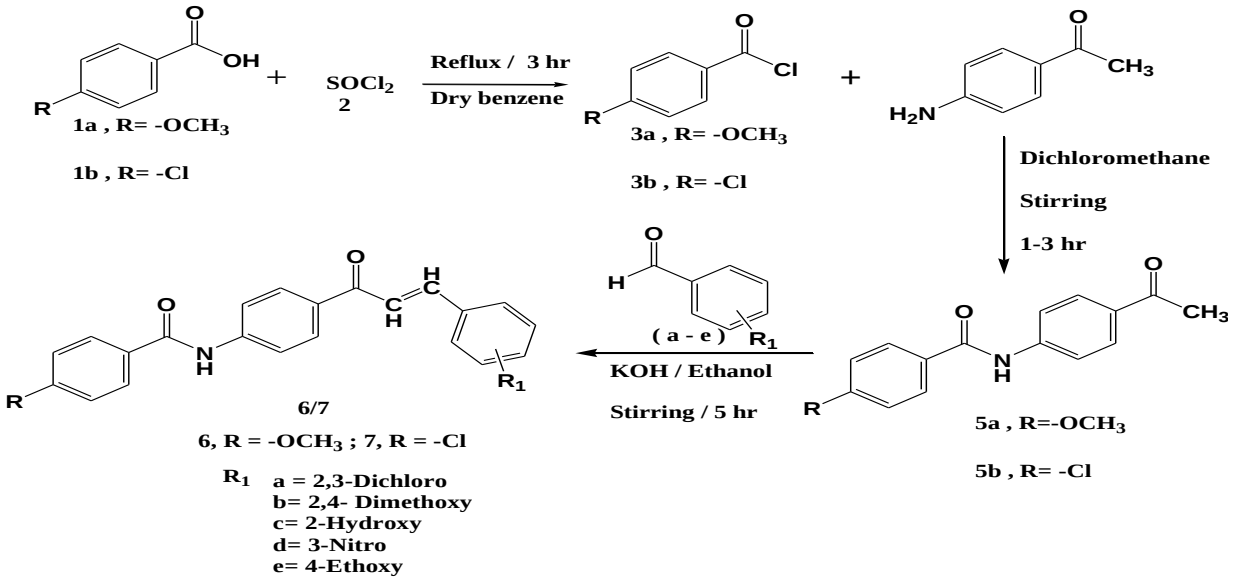
Scheme 1. Reaction scheme for synthesis of chalcone derivatives **6 (a-e)** and **7 (a-e)**



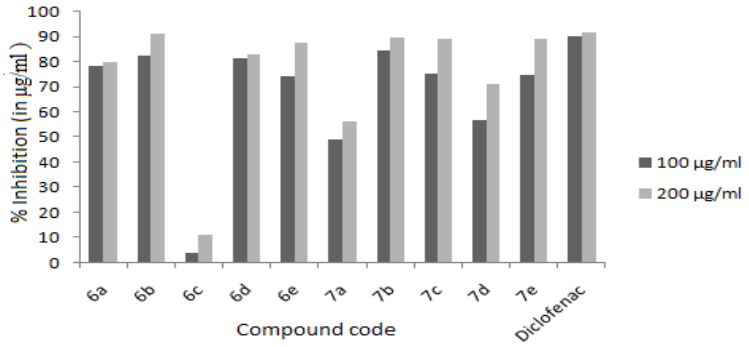


Figure 1 *In vitro* anti-inflammatory activity of chalcone derivatives **6 (a-e)** and **7 (a-e)**

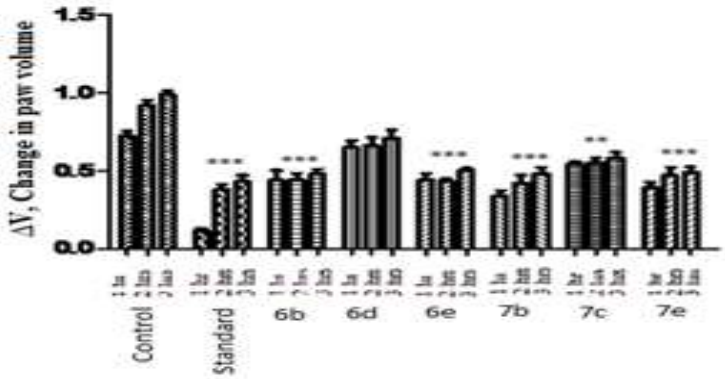


Figure 2 *In vivo* anti-inflammatory activity of chalcone derivatives by carrageenan induced rat paw oedema method

Table 1 Characterization data of chalcone derivatives 6 (a-e) and 7 (a-e)

Compound code	Mol. Formula	M.P (°C)	Yield (%)	Elemental analysis (%) Calculated (found)		
				C	H	N
6a	$C_{23}H_{17}O_3Cl_2N$	262-266	89	_	_	_
6b	$C_{25}H_{23}O_5N$	208-210	88	71.9 (71.8)	5.5 (5.6)	3.35 (3.45)
6c	$C_{23}H_{19}O_4N$	154-160	48	_	_	_
6d	$C_{23}H_{18}O_5N_2$	204-208	51	_	_	_
6e	$C_{25}H_{23}O_4N$	180-182	77	_	_	_
7a	$C_{22}H_{14}O_2Cl_3$	120-124	92	_	_	_
7b	$C_{24}H_{20}O_4NCl$	182-186	84	68.4 (68.2)	4.75 (4.71)	3.32 (3.27)
7c	$C_{22}H_{16}O_3NCl$	218-220	59	_	_	_
7d	$C_{22}H_{15}O_4N_2Cl$	202-206	74	_	_	_
7e	$C_{24}H_{20}O_3NCl$	220-222	83	_	_	_

Table 2 Physical parameters of the compounds

Compound code	Mol. Wt.	clogP[a]	R_M	TPSA[b]	nrotb[c]	SMV[d]
Lipinski[e]	$\leq$ 500	$\leq$ 5.0				
6b	417.46	4.39	0.34	73.87	8	381.28
6d	402.40	4.69	0.36	101.22	7	353.52
6e	401.46	5.19	0.07	64.63	8	372.54
7b	421.88	5.24	0.15	64.63	7	369.27
7c	377.82	5.14	0.19	66.39	5	326.20
7e	405.88	5.81	-0.16	55.40	7	360.53

a= clogP value, b= topological polar surface area, c= number of rotatable bonds, d= molar volume (A^3), e= Lipinski's rule of 5 for pharmaceuticals.

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