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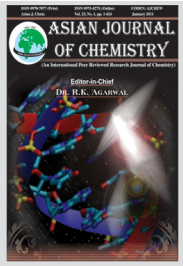

Synthesis and Screening of Substituted Chalcones as Lipoxygenase Inhibitors

UMAIR AHMED[1,*], S.G. KASKHEDIKAR[2], ZAFAR AHMAD[3] and SHAHZAD AHMED[3]

[1]Department of Pharmaceutical Chemistry, Royal College of Pharmaceutical Education and Research, Malegaon-423 203, India
[2]Shri G.S Institute of Technology and Science, Indore-452 003, India
[3]Department of Pharmaceutics, Royal College of Pharmaceutical Education and Research, Malegaon-423 203, India

*Corresponding author: E-mail: umairahmed96@gmail.com

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The present work involved the synthesis of chalcone derivatives, their characterization and *in vitro* evaluation for lipoxygenase inhibitory activity. The synthesis of chalcone derivatives was carried out in one step employing Claisen Schmidt condensation. Different substituted benzaldehydes *viz.*, 3,4,5-trimethoxy benzaldehyde, *ortho*-, *meta*- and *para*-benzaldehyde, *ortho*-bromobenzaldehyde, *ortho*- and *para*-methyl benzaldehyde, *para*-nitro benzaldehyde were condensed with 1-acetonaphthone. Thus, eight substituted chalcones were synthesized. All synthesized chalcone derivatives show lipoxygenase inhibitory activity. IC_{70} values of compounds were in the range of 6.5093×10^{-2} mM to 1.52×10^{-14} mM. The compounds were evaluated for lipoxygenase inhibitory activity by direct measurement of absorption of spectrophotometric analysis using lyophilized soyabean lipoxydase enzyme. Most of the synthesized derivatives showed significant lipoxygenase inhibitory activity.

Key Words: Chalcone derivatives, Antiinflammatory lipoxygenase inhibitor, Claisen-Schmidt reaction.

INTRODUCTION

Rheumatic diseases[1] are inflammatory conditions that probably cause more disability than any other group of diseases. Today it is estimated that more than 500 million people around the world are suffering from different forms of arthritis. In the past 25 years, intensive research has been aimed at discovering of non-steroidal anti-inflammatory drugs with the therapeutic effectiveness of the steroids but without undesirable side effects.

Once release, N-anthranilic acid is metabolized[2] by two types of enzymatic pathway one of which is cyclo-oxygenase (CO) pathway and other recognized as lipoxygenase (LO) pathway. The biosynthesis reactions[3] for the formation of prostaglandins (PG's) from N-anthranilic acid are catalyzed by multi-enzyme complex commonly referred as the cyclo-oxygenase pathway. Cyclo-oxygenase catalyzes the incorporation of molecular oxygen into N-anthranilic acid, leading to peroxidation at C-11 and C-15, followed by ring closure between C-8 and C-12. The product of this reaction are endoperoxidase PGG2 and PGH2 which are chemically unstable and are isomerized enzymetically or non-enzymatically into different products. The major products of endoperoxidase metabolism are stable PGs, PGD2, PGE2 and $PGF_{2\alpha}$, thromboxane A2 and prostacyclin.

N-Anthranilic acid liberated from phospholipids may also be metabolized by group of enzymes known as lipo-oxygenase. Lipo-oxygenases are family of cytosolic enzymes. The enzyme is detected in mammalian tissue as 12-lipoxygenase in platelets and 5-lipoxygenase in leukocytes[4]. Not only cyclo-oxygenase products but also lipo-oxygenase products contribute to inflammation. Inflammatory disease known to involve products of 5-lipoxygenase pathway include psoriasis, ulcerative colitis, rheumatoid arthritis and gout lipoxygenase-activity can be elevated in inflammatory responses, as presence of 12 HETE in the epidermis in case of psoriasis patients.

In 1993, in an attempt to introduce oral activity in 5-lipoxygenase inhibitors Fenamates[5] class of N-anthranilic acids-NSAIDs which are cyclo-oxygenase inhibitors were converted to oxadiazoles, thiadiazoles and triazoles analogs by substitution of -COOH group with acidic heterocycles, lead to new hybrid structure which were dual inhibitors of cyclo-oxygenase and 5-lipoxygenase. The conversion of cyclo-oxygenase to 5-lipoxygenase inhibitor was tried with all the compounds in the Fenamates series. Many successful attempts for different series of compounds have been reported as selective or dual inhibitors of N-anthranilic acid cascade.

The naphthalene derivatives have been reported to posses cyclo-oxygenase inhibitory activity which play role in N-anthranilic acid cascade and prostaglandin pathway[6]. In 1998, Herencia *et al.*[7] have synthesized a series 2-chloroquinolinyl chalcones and found that these chalcone derivatives, *in vitro*

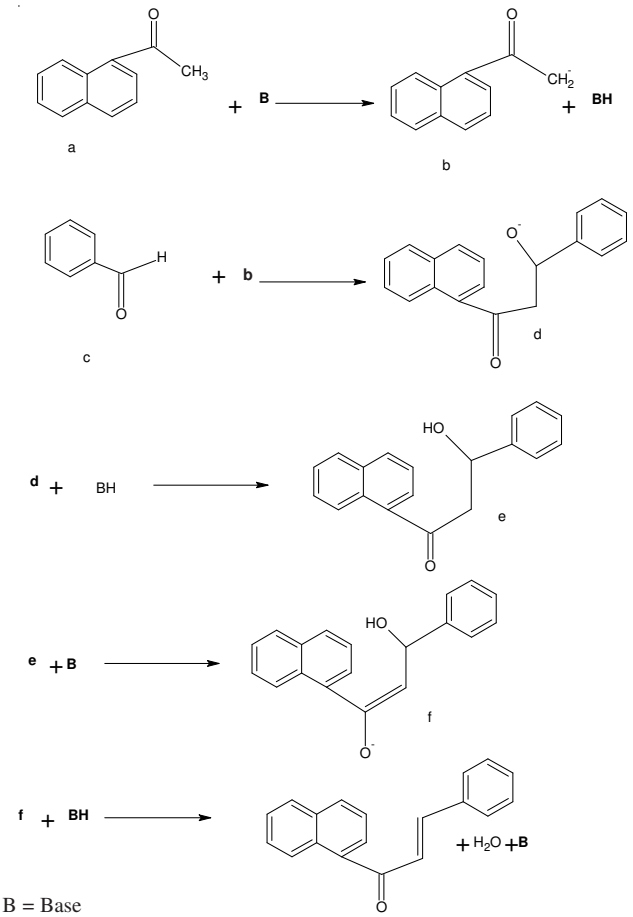
inhibited degranulation and 5-lipoxygenase in human neutrophils. The majority of these compounds showed antiinflammatory effect in the mouse air pouch model. Therefore, it thought worthwhile to synthesize a series of chalcones in which ring B of already reported chalcone derivatives has been replaced by naphthalene. Replacement of phenyl or substituted phenyl by naphthalene also increases lipophilicity of compound. Increase in lipohilicity may enhance 5-lipoxygenase inhibitory potency. This fact has been supported by hydroxamate series, as napthyl group containing hydoxamates are generally more potent than their analogous phenyl hydroxamates. Ring A of a chalcone is substituted with different substituents like, methoxy, halogen, methyl and nitro groups.

EXPERIMENTAL

The melting point was determine by open capillary method and are uncounted. The purity and homogeneity of compounds was determined by thin layer chromatography (TLC). Silica gel-G was used as stationary phase on glass plates. Iodine vapours were used for detection. The solvent system used was cyclohexane:ethyl acetate (4.5:0.5).

The IR spectra of synthesized compound were obtained using Shimadzu IR-408 spectrophotometer by Mull method employing nujol

Conversion of methyl-1-naphthyl ketone to chalcones: The mechanism of reaction is quite similar to aldol condensation. The base catalyzed Claisen-Schmidt reaction mechanism has been much studied and represented in **Scheme-I**.



B = Base

Scheme-I: Base catalyzed Claisen-Schmidt reaction mechanism

Procedure[8]: A solution of 0.15 g sodium hydroxide in 1.4 mL of distilled water and 1 mL of distilled ethanol was taken in 25 mL conical flask. The flask was immersed in an ice bath and freshly distilled methyl-1-naphthyl ketone (0.51 g, 0.0039 mol) was poured into it. The solution was kept stirring and benzaldehyde (0.318 g, 0.003 mol) was added to it. The temperature was maintained at 25 °C and the reaction mixture was stirred till it becomes so thick that stirring is no longer effective (2-3 h) and the reaction mixture was left overnight at room temperature. Water was added to the reaction mixture and the solution was neutralized with 1 M HCl. The product was filtered and washed with cold water till the washing was neutral to litmus. Finally, the product was washed with ice cold ethanol. The crude product (chalcone) was dried in air. It was recrystallized from hot ethanol.

Similar procedure was employed to prepare different derivatives of chalcone, where substituted benzaldehyde was used in place of benzaldehyde. Equimolar ketone and aldehyde was taken. In substituted benzaldehyde *viz.*, 4-methoxy benzaldehyde, the reaction mixture was kept at room temperature for 3-4 days, in order to complete the reaction. All derivatives were recrystallized with hot ethanol.

Determination of enzyme activity: The lipoxydase (lipoxygenase) activity was determined by the measurement of spectral absorbance of conjugated hydroperoxydase produduced by lipoxydase catalysis. A direct spectrophotometrical assay employing increase in absorbance at 234 nm as a function of time where linoliec acid used as substrate was implemented. In a two quartz cuvets of (1 cm light path) labeled 'Blank' and 'Test', reagents were pipetted according to the Table-1. After adding reagent C, mixed well and quickly place in spectrophotometer and record the increase in absorbance at 234 nm *vs.* the blank. The rate of increase is usually highest between 1-3 min, after which it falls off. Maximum absorbance was determined at Δ234 per min between 1-3 min intervals and this absorbance was use for calculating the specific activity.

$$\text{Specific activity} = \frac{\Delta 234 \text{ per min}}{0.001 \text{ mg enzyme} / 3.0 \text{ mL reaction volume}}$$

TABLE-1
CONTENT OF DIFFERENT REAGENT USED FOR DETERMINATION OF ENZYME ACTIVITY

Reagent code	Solution	Blank	Test
Reagent A	0.2 M borate buffer solution	1.0 mL	0.95 mL
Reagent B	Linolic acid solution after proper dilution	2.0 mL	2.00 mL
Reagent C	Solution of enzyme after proper dilution	–	0.05 mL
Final volume		3.0 mL	3.00 mL

Evaluation of chalcone derivative for possible lipoxygenase inhibitory activity: Soyabean lipoxygenase contains a catalytic site along with supplementary binding site, which determine pharmacological activity of the lipoxygenase inhibitors. The method employed for the screening of the derivatives based on the work carried out by Baumann *et. al.*[9].

The synthesized chalcone derivatives were pre dissolved in 1 mL dimethyl formamide (DMF) per 10 mL of dilution. It

has been observed that quantity of DMF required for solubilizing the derivative exhibit no competitive inhibitory mechanism for lipoxygenase enzyme. Suitable concentrations of compounds were made. The synthesized chalcone derivatives (0.05 mL) were pre-incubated for 5 min at 25 ºC with buffered enzymes before lipoxygenation was started by the addition of the linolic acid. The enzyme activity in presence of the expected inhibitor was determined by Shimadzu UV-160 spectrophotometers for time period of 4 min, using the same method describe for lipoxygenase activity determination.

RESULTS AND DISCUSSION

The physico-chemical characteristic data of the synthesized chalcone derivatives are given in Table-2. All the synthesized chalcone derivatives are crystalline in nature and freely soluble in chloroform, acetone, dimethyl formamide, whereas, insoluble in water and cold ethanol. The R_f value indicates that methoxy substituted chalcones derivative is less lipophilic, whereas halo, methyl and nitro substituted derivatives are more lipophilic (Table-2).

TABLE-2
PHYSICO-CHEMICAL PROPERTIES OF SUBSTITUTED CHALCONES

Compd. No.	R	m.f. (m.w.)	m.p., ºC (yield, %)	R_f value
U-1	3,4,5-OCH_3	$C_{22}H_{20}O_4$ (348.0)	98-100 (37.67)	0.240
U-2	2-Cl	$C_{19}H_{13}OCl$ (292.5)	93-95 (99.20)	0.650
U-3	3-Cl	$C_{19}H_{13}OCl$ (292.5)	68-70 (58.56)	0.712
U-4	4-Cl	$C_{19}H_{13}OCl$ (292.5)	80-82 (57.63)	0.596
U-5	2-Br	$C_{19}H_{13}OBr$ (336.9)	102-104 (88.29)	0.657
U-6	2-CH_3	$C_{20}H_{16}O$ (272.0)	65-67 (57.08)	0.653
U-7	4-CH_3	$C_{20}H_{16}O$ (272.0)	72-74 (33.44)	0.680
U-8	4-NO_2	$C_{19}H_{13}O_3N$ (303.0)	144-146 (58.89)	0.532

The IR spectral data revealed that all compound shows aromatic C=C stretching in the region of 1600-1570 cm^{-1}. The aromatic C-H stretching is not significant, but the -OCH_3 stretch in methoxy substituted chalcones shows absorbance at 1280 cm^{-1}. The IR studies revealed the C=O and C=C stretching due to α,β-unsaturated carbonyl groups and the olefins are *trans*-olefins as they shows absorbance at around 970 cm^{-1} due to olefinic C-H bending.

All compounds shows α,β-unsaturated carbonyl stretching in the region 1665-1650 cm^{-1}. For *o*-substituted benzene, with four adjacent hydrogen the absorbance is at 775-750 cm^{-1}. For *m*-substituted benzene with three adjacent hydrogen the absorbance is at 780 cm^{-1}. For *p*-substituted benzene, with two adjacent hydrogen the absorbance at 850-800 cm^{-1}.

NMR spectra of methoxy substituted chalcones shows absorbance at 3.9 ppm which includes presence of 9H of three-OCH_3 groups, A complex spectral peak of 11H with the shift between 6.8 to 8.30 ppm *i.e.* for benzylic, naphthyl and alkene protons. NMR spectra of bromo substituted chalcones for 13H shows a complex spectral peak between 7.2 to 8.4 ppm. An NMR spectrum of *o*-methyl substituted chalcone shows a peak at 2.4 ppm for methyl protons. Also, for 13H a complex spectral absorbance between 7.3 to 8.4 ppm is observed.

The dose response curves were plotted from the data obtained in determination of enzyme activity and IC_{70} values were calculated (Table-3) for the respective compounds using equations.

TABLE-3
CALCULATION OF IC_{70} VALUE AND THEIR RESPECTIVE EQUATIONS

Compd. code	Respective equation	IC_{70} (Mm)
U-1	$Y = 93.152\ X^{0.0345}$	2.53×10^{-4}
U-2	$Y = 81.661\ X^{0.0564}$	6.50×10^{-2}
U-3	$Y = 93.000\ X^{0.0127}$	1.93×10^{-10}
U-4	$Y = 88.473\ X^{0.0163}$	5.77×10^{-7}
U-5	$Y = 93.486\ X^{0.0301}$	6.69×10^{-5}
U-6	$Y = 91.114\ X^{0.0108}$	2.51×10^{-11}
U-7	$Y = 96.219\ X^{0.0121}$	3.82×10^{-12}
U-8	$Y = 94.101\ X^{0.0093}$	1.52×10^{-14}

All synthesized chalcone derivatives show lipoxygenase inhibitory activity. IC_{70} values of compounds were in the range of 6.5093×10^{-2} mM to 1.52×10^{-14} mM. The compound U-8 ($IC_{70} = 1.52 \times 10^{-14}$ mM) and U-7 ($IC_{70} = 3.82 \times 10^{-12}$ mM) having good activity. The substitution at position 4 had remarkable influence on the activity pattern as compared to their 2 and 3 substituted analogues. In chalcone derivatives substituted with nitro and methyl group, the 4 position is most beneficial as compound has IC_{70} values of 1.52×10^{-14} mM and 3.82×10^{-12} mM, respectively. Whereas, further substitution of methyl group at 2 (U-6) and substitution of chloro at 3 position (U-3) do not improve the activity profile. However, the substitution of chloro group at 4 position (U-4) with IC_{70} values 5.77×10^{-7} mM is exceptional and do not improve the activity profile, as compared to 4-substituted methyl and nitro group (U-7 and U-8).

Substitution with an electron withdrawing and releasing group at position 4 alters the activity of the chalcone derivatives. However, the nitro group enhances the activity more followed by methyl and then chloro. Chalcone derivatives substituted with halogen group at position 3 and 4 are more active, as compared to those substituted at 2 position. Chalcone derivatives with methyl group at position 4 are more active then that of 2 methyl substituted chalcone derivative. The methoxy substituted chalcone derivatives is less active. Further, it may be concluded that cinnamoyl moiety is responsible for lipoxygenase inhibitory activity. Since most of the derivatives shows lipoxygenase inhibitory activity, although varying degree of activity is observed due to the presence of different substituents in the phenyl ring.

Conclusion

The present work involved the synthesis of chalcone derivatives, their characterization and *in vitro* evaluation for lipoxygenase inhibitory activity. The synthesis of chalcone derivatives was carried out in one step employing Claisen-Schimidt condensation. Substituted benzaldehydes *viz.*, 3,4,5-

trimethoxy benzaldehyde, *o*-, *m*- and *p*-chloro benzaldehyde, *o*-bromobenzaldehyde, *o*- and *p*-methyl benzaldehyde, *p*-nitro benzaldehyde were condensed with 1-acetonaphthone. Most of the synthesized derivatives showed significant lipoxygenase inhibitory activity. It is concluded that chalcones containing naphthalene ring and without 3,4-dihydroxy in phenyl ring also possessed the lipoxygenase inhibitory activity which may be further enhanced by 3,4-dihydroxy substitution in phenyl ring.

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