Synthesis and preliminary evaluation of a focused chalcone library for anti-inflammatory activity

Ranganathan Balasubramanian*[,1], Hiba Iqbal[1], Rachana Vijaya Gopal[1] and C Baby[2]

[1]Department of Pharmaceutical Chemistry, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham University, Health Sciences Campus, Kochi-682041, Kerala, India
[2] Sophisticated Analytical Instrumentation Facility, Indian Institute of Technology, Madras, Chennai-600036, Tamil Nadu, India

ABSTRACT

A series of monosubstituted chalcones with potential anti-inflammatory activity have been rationally designed leading to a focused library of ligands **4a-j** with ring-A substitution. Subsequent to drug-likeness and druggability assessments, the library members were synthesized in optimal yields and their structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. Screening for anti-inflammatory activity revealed that most of the compounds were quite effective in containing inflammation with compound **4b** being the most promising derivative.

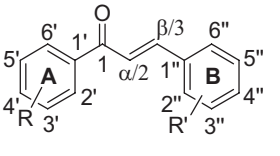
Keywords: Chalcones, structure-activity relationship, drug-likeness, anti-inflammatory activity.

INTRODUCTION

Chalcones are natural or synthetic 1,3-diaryl-2-propen-1-ones **(1)** that may exist in *cis* and *trans* isomeric forms, of which the latter is thermodynamically stable. Illustrated by the general structure in Fig. 1, they contain an open-chain flavonoid skeleton in which two aromatic rings (ring-A and ring-B) are linked by a three-carbon α,β-unsaturated carbonyl system. Majority of the naturally occurring chalcones contain benzene rings with hydroxy, methoxy and alkenyl groups as their aryl substituents. Besides such substituted phenyl groups, the synthetically derived chalcones may carry heterocyclic and condensed ring systems as their aryl substituents.[1]

Chalcones continue to attract a lot of interest in both academia and industry mainly due to their enormous pharmacological potential. They have been reported to possess an array of useful therapeutic properties, including anti-inflammatory,[2,3] anti-microbial,[4] anti-oxidant,[5] and anti-cancer[6] activities among others. The α,β-unsaturated ketone moiety is considered the key pharmacophoric feature as its partial or full removal leads to a loss of bioactivity. Anti-inflammatory activity of chalcones is manifested by their interaction with a number of targets;[7] some of which include inducible nitric oxide synthase (iNOS),[8] nuclear factor-κB (NF-κB),[9–11] heme oxygenase (HO)[12–14] and cyclooxygenase (COX).[15]

Most of these reports describe the molecular mechanism of anti-inflammatory action of one or a very limited number of chalcones exhibiting complex substitution patterns on their aromatic rings. Under such a situation, it is very difficult to derive useful structure-activity relationship (SAR) data. In an effort to systematically probe the structural features required



R & R' are variable; 1,3-diaryl-2-propen-1-ones **(1)**

Figure 1: General chemical structure of chalcones.

DOI: 10.5530/ijper.47.4.5

Address for correspondence
Dr. B. Ranganathan
Associate Professor, Department of Pharmaceutical Chemistry, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham University, Health Sciences Campus, Kochi-682041, Kerala, India
Tel. No: 0484-2851234 Extn. 7919
Fax: 0484-2802020
E-Mail: ranga.balasubramanian@gmail.com

www.ijper.org

for anti-inflammatory activity, we have embarked on the synthesis of small libraries of chalcones. Each library would focus on one particular fragment of the molecule and comprise of members which are structural and/or functional variants of that fragment. The results obtained from each such library can then be meaningfully interpreted to decipher SAR information. The present study, wherein we describe the synthesis and preliminary biological evaluation of a library of ring-A monosubstituted chalcones, is an outcome of this exercise. Given the paucity of systematic SAR information on this scaffold with respect to inflammation coupled with the lack of safe anti-inflammatory agents in the clinic, such a study is very relevant in an attempt to bridge this gap.

MATERIALS AND METHODS

The drug-likeness assessment involving the calculation of topological polar surface area (TPSA) and the Lipinski parameters were carried out using the high-speed molecular properties calculator which is a free module in the MolSoft software package. The ADMET properties were predicted using ADMETox descriptors tool in Discovery Studio 2.0, an Accelrys computational package.

Design of ligands

The main aspect in the design of ligands for this study was the choice of substituents to be appended to the acetophenic phenyl group (ring-A) in the parent chalcone (1,3-diphenyl-2-propen-1-one, **4a**). It was decided to generate this library **(4a-j)** by introducing one substituent at a time in such a way that ring-A would be 2'- (*o*-), 3'- (*m*-) or 4'- (*p*-)-substituted while the alkenyl phenyl group (ring-B) would remain unsubstituted. Amino, hydroxy, methoxy (+*M*); methyl (+*I*); nitro (-*M*) and bromo (-*I*) groups were chosen for their wide-ranging electronic properties while the phenyl substituent was picked to probe the importance of sterics.[16] All three regioisomeric ring-A monosubstituted bromochalcones were included in the designed set to investigate the role of the substitution pattern in the ring. The ready availability of the starting materials and their cost were other factors that dictated the choice of the library members. The target chalcones **4a-j**, assembled from the respective commercially available acetophenone precursors **2a-j**, are listed in Table No. 1.

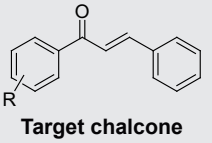
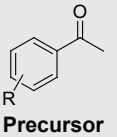
Drug-likeness assessment

Compounds **4a-j** were then screened by employing Lipinski's rule of five as a filter. This is a heuristic approach for predicting drug-likeness which states that molecules having molecular weight > 500 Daltons, octanol-water partition coefficient logP > 5, hydrogen bond donors > 5 and hydrogen bond acceptors > 10 will likely have poor absorption or permeation. The original set of empirical rules[17] stated above have spawned many extensions to include parameters like TPSA,[18] which is expected to be not greater than 140 Å^2. These empirical drug-likeness predictors of all the ten chalcones under study are presented in Table No. 2.

ADMET prediction

In addition to the assessment of drug-likeness, the druggability of the series was evaluated through another screen that predicts the absorption, distribution, metabolism, excretion and/or toxicity (ADMET) properties like solubility, human intestinal absorption (HIA), plasma protein

Table 1: List of Target Chalcones and Precursors

 Target chalcone	 Precursor	R
Unsubstituted chalcone **(4a)**	Acetophenone **(2a)**	H
2'-Hydroxychalcone **(4b)**	2'-Hydroxyacetophenone **(2b)**	2'-OH
2'-Methylchalcone **(4c)**	2'-Methylacetophenone **(2c)**	2'-CH_3
2'-Bromochalcone **(4d)**	2'-Bromoacetophenone **(2d)**	2'-Br
2'-Aminochalcone **(4e)**	2'-Aminoacetophenone **(2e)**	2'-NH_2
3'-Nitrochalcone **(4f)**	3'-Nitroacetophenone **(2f)**	3'-NO_2
3'-Bromochalcone **(4g)**	3'-Bromoacetophenone **(2g)**	3'-Br
4'-Methoxychalcone **(4h)**	4'-Methoxyacetophenone **(2h)**	4'-OCH_3
4'-Phenylchalcone **(4i)**	4'-Phenylacetophenone **(2i)**	4'-Ph
4'-Bromochalcone **(4j)**	4'-Bromoacetophenone **(2j)**	4'-Br

Monosubstituents appended such that ring-A would be 2'- (*o*-), 3'- (*m*-) or 4'- (*p*-) substituted

binding (PPB), blood brain barrier (BBB) penetrability, hepatotoxicity and the ability to bind to cytochrome P450 enzymes. The results for the target chalcone library and the reference compound ibuprofen, obtained as scores of each parameter, are summarized in Table No. 3 wherein a detailed key is provided for the ready interpretation of the scores.

Chemistry experimental section

All commercial and analytical reagents were used as provided unless otherwise indicated. Ibuprofen standard used for testing the anti-inflammatory activity was obtained as a gift from Porus Labs Pvt. Ltd., Hyderabad. TLC was performed on silica gel G (40 μm particle size)-coated glass plates and spots visualized by UV or by exposure to iodine vapours. Melting points were determined using a Roy Capillary Melting Point Apparatus and are uncorrected. Boiling points were determined using Thiele's tube. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 or Bruker AV III 500 MHz spectrometer. Proton and carbon chemical shifts are reported in ppm from an internal standard of residual $CHCl_3$ (7.26 ppm & 77.16 ppm respectively). Proton chemical data are reported as follows: chemical shift, multiplicity (ovlp = overlapping, s = single, d = doublet, t = triplet, m = multiplet), coupling constant and integration. IR spectra were recorded on a Shimadzu Affinity-1 FT-IR spectrophotometer. LC-MS chromatograms and spectra were obtained using a Thermo Finnigan and/or Agilent Technologies instrument using a combination of either APCI-PDA or ESI-DAD as the mode of ionization and type of detector respectively.

Table 2: The Lipinski Parameters and TPSA of the Target Chalcones

Compound	ADME formula (whole molecule)	ADME weight g/mol (whole molecule)	ADME H-bond acceptor(s) (whole molecule)	ADME H-bond donor(s) (whole molecule)	ADME LogP (whole molecule)	TPSA Å² (whole molecule)
4a	$C_{15}H_{12}O$	208.09	1	0	3.95	17.07
4b[a]	$C_{15}H_{12}O_2$	224.08	2	1	4.09	37.30
4c	$C_{15}H_{13}NO$	223.10	1	2	3.59	43.09
4d	$C_{15}H_{11}BrO$	286.00	1	0	4.77	17.07
4e	$C_{15}H_{13}NO$	223.10	1	2	3.59	43.09
4f	$C_{15}H_{11}NO_3$	253.07	3	0	3.93	62.89
4g	$C_{15}H_{11}BrO$	286.00	1	0	4.91	17.07
4h[a]	$C_{16}H_{14}O_2$	238.10	2	0	4.00	26.30
4i[a]	$C_{12}H_{16}O$	284.12	1	0	5.93	17.07
4j	$C_{15}H_{11}BrO$	286.00	1	0	4.84	17.07
Ibuprofen	$C_{13}H_{18}O_2$	206.13	2	1	3.38	37.30

[a] *=parameters taken from ref. no. 16*

All our ligands are drug-like and would be amenable to oral administration in humans

Table 3: ADMET Properties of the Target Chalcones and Ibuprofen

Compound	BBB level	Absorption level	Solubility level	Hepatotoxicity	CYP2D6 inhibition	PPB
4a	0	0	2	1	0	2
4b	1	0	3	1	0	2
4c	0	0	2	1	1	2
4d	0	0	2	1	1	2
4e	1	0	3	1	1	2
4f	1	0	2	1	1	1
4g	0	0	2	1	1	2
4h	1	0	2	1	1	2
4i	0	0	2	1	1	2
4j	1	0	2	1	1	2
Ibuprofen	1	0	3	1	0	2

BBB: 0- very high penetration; 1- high penetration; 2- medium penetration; 3- low penetration; 4- undefined. Absorption level: 0-good absorption; 1- moderate absorption; 2- poor absorption; 3- very low absorption. Solubility level: 0- very high solubility; 1- high solubility; 2- medium solubility; 3- low solubility; 4- undefined. Hepatotoxicity: 0- non-toxic, unlikely to cause dose- dependent liver injuries; 1- toxic, likely to cause dose-dependent liver injuries. CYP2D6 Inhibition: 0- not a likely inhibitor; 1- potential inhibitor. PPB Level: 0- binding is < 90% (No markers flagged and AlogP98 < 4.0); 1- binding is ≥ 90% (flagged at 90% or AlogP98 ≥ 4.0); 2- binding is ≥ 95% (flagged at 95% or AlogP98 ≥ 5.0).

General procedures for chalcone synthesis

Method A

To a solution of substituted acetophenone (10 mmol) in rectified spirit (30 ml) was added benzaldehyde (1.01 ml, 10 mmol) followed by an aqueous solution of 10% KOH (10 ml). The mixture was stirred and kept overnight at room temperature. The contents of the reaction mixture were poured into crushed ice and neutralized with 0.1 N dilute HCl. The precipitated chalcone derivative was filtered off and recrystallized from rectified spirit.

Method B

To a vigorously stirring mixture of benzaldehyde (1.01 ml, 10 mmol) and substituted acetophenone (10 mmol) in rectified spirit (10 ml) was added dropwise over 30 m an aqueous solution of 10% NaOH (10 ml). The reaction mixture was maintained between 20–25°C using a cold water bath and stirring was continued for a further 5 h. It was then neutralized using 0.1 N dilute HCl whereby precipitation occurred. The crude chalcone derivative was filtered off, dried in air and recrystallized from rectified spirit.

Method C

Equal quantities of benzaldehyde (0.10 ml, 1 mmol) and substituted acetophenone (1 mmol) were mixed and dissolved in rectified spirit (3 ml) followed by the gradual addition of aqueous KOH (3 mmol). The contents of the flask were swirled until complete dissolution and the entire reaction mixture was then irradiated for 7–8 m at 160 W under microwave irradiation. The contents were poured into crushed ice and acidified with 0.1 N dilute HCl. The chalcone derivative that precipitated out was filtered off and recrystallized from rectified spirit.

The three methods of syntheses described above are illustrated in Fig. 2 while the yields, appearance, R_f values and melting points of compounds **4a-4j** are listed in Table No. 4. Further, their spectral characterization including FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectrometric data is reported in Table No. 5.

Testing of anti-inflammatory activity

In a typical experiment,[19] the standard drug (ibuprofen) and test compounds were dissolved in minimum amount of DMF and diluted with phosphate buffer saline (0.2 M, pH 7.4) in such a way that concentration of DMF in all solutions was less than 2.5%. Test solution was mixed with bovine serum albumin (BSA) solution (2 ml, 2 mmol) in phosphate buffer saline and placed in an incubator at 27 ± 1°C for 15 m following which denaturation was induced by warming and subsequently maintaining the reaction mixture at 60 ± 1°C in a water bath for 10 m. After cooling, the turbidity was measured at 660 nm with UV visible spectrophotometer and the percentage inhibition effected in the process of denaturation was calculated from control where no drug

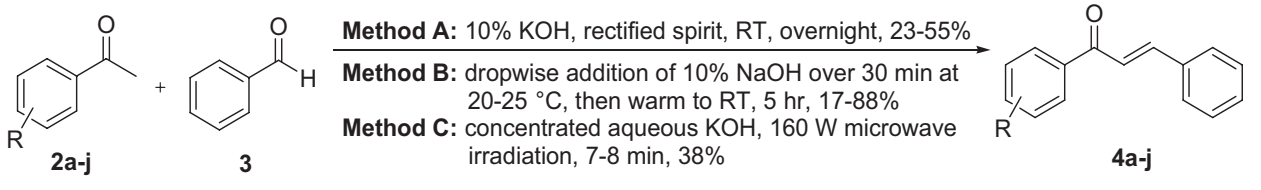


Figure 2: Methods for the synthesis of target chalcones.

Table 4: Physical Data of Synthesized Compounds 4a-4j

Compound	Method of synthesis	Appearance	Yield %	R_f	MP °C
4a	Method C	Yellow solid	38	R_f^b = 0.6	50–52
4b	Method A	Off-white solid	25	R_f^b = 0.6	68–73
4c	Method A	Yellow liquid	23	R_f^c = 0.61	NA[e]
4d	Method A	Yellow liquid	33	R_f^c = 0.65	NA[f]
4e	Method A	Dark yellow flakes	26	R_f^c = 0.7	50–58
4f	Method A	Dark brown solid	55	R_f^d = 0.5	115–118
4g	Method B	Cream white crystals	17	R_f^c = 0.61	70–74
4h	Method B	Cream white needles	60	R_f^b = 0.4	70–75
4i	Method A	Light yellow crystals	33	R_f^b = 0.56	120–122
4j	Method B	Cream white flakes	88	R_f^b = 0.7	70–78

R_f^b = 10% EtOAc/hexanes; R_f^c = 10% EtOAc/cyclohexane; R_f^d = 20% EtOAc/cyclohexane; [e] = BP 165°C; [f] = BP 75°C
Recrystallization solvent: rectified spirit

Table 5: Spectral Characterization Data of Synthesized Compounds

Compound	IR(KBr) cm^{-1}	^{1}H NMR	^{13}C NMR	Mass data
4a	3057 (arom.[g] -CH str.[h]), 1662 (α,β-unsaturated keto group, C=O str.[h]), 1604 (-C=CH arom.[g] -CH str.[h], C=C str.[h]), 1446 (saturated alkene in plane), 991 (oop[i], -CH bend.[j] vibration of alkene), 751 (arom.[g] bend.[j])	($CDCl_3$, 500 MHz) δ 8.02 (dd, *J* = 7, 8.5 Hz, 1H), 7.81 (d, *J* = 15.5 Hz, 1H), 7.62-7.65 (m, 2H), 7.56-7.60 (m, 1H), 7.53 (d, *J* = 15.5 Hz, 1H), 7.49 (d, *J* = 7.5 Hz, 2H), 7.40-7.44 (m, 3H)	($CDCl_3$, 125 MHz) δ 190.6, 144.9, 138.3, 135.0, 132.8, 130.6, 129.0, 128.6, 128.4, 122.2	ESI MS [*m/z*, relative abundance] [209 $(M+H)^+$, 100]
4b	3356 (intramolecular H-bonded, -OH str.[h]), 3057 (arom.[g] -CH str.[h]), 1689 (α,β-unsaturated keto group, -C=O str.[h]), 1462 (C=C ring str.[h]), 1224 (C-O str.[h] of C-OH), 763 (1,2 disubstitution)	($CDCl_3$, 400 MHz) δ 7.94 (d, *J* = 8 Hz, 1H), 7.38-7.54 (m, 6H), 7.06-7.08 (m, 2H), 5.49 (dd, *J* = 2.8, 16 Hz, 1H), 3.10 (t, *J* = 15.2 Hz, 1H), 2.91 (d, *J* = 16.8 Hz, 1H)	($CDCl_3$, 100 MHz) δ 192.0, 165.4, 161.6, 138.8, 136.2, 133.7, 129.7, 128.8, 128.7, 127.1, 126.2, 121.6, 121.0, 118.1, 79.6	APCI MS [*m/z*, relative abundance] [225 $(M+H)^+$, 100], [179 $(225-H_2O)^+$, 3], [147 $(C_{14}H_{16})^+$, 8], [121 $(C_{12}H_{14})^+$, 20]
4c	3059 (arom.[g] -CH str.[h]), 2930 (aliphatic -CH str.[h]), 1641 (α,β-unsaturated keto group, -C=O str.[h]), 1600 (=CH arom.[g] str.[h], C=C ring str.[h]), 754 (1,2 disubstitution)	($CDCl_3$, 400 MHz) δ 7.54-7.58 (m, 2H), 7.51 (d, *J* = 1.6 Hz, 1H), 7.47 (d, *J* = 16.4 Hz, 1H), 7.37-7.42 (m, 4H), 7.29-7.30 (m, 1H), 7.27-7.28 (m, 1H), 7.14 (d, *J* = 16 Hz, 1H), 2.45 (s, 3H)	($CDCl_3$, 100 MHz) δ 196.5, 145.8, 139.0, 136.9, 134.6, 131.3, 130.6, 130.5, 129.0, 128.4, 128.1, 126.7, 125.5, 20.2	ESI MS [*m/z*, relative abundance] [223 $(M+H)^+$, 100]
4d	3059 (arom.[g] -CH str.[h]), 1649 (α,β-unsaturated keto group, -C=O str.[h]), 1599 (=CH arom.[g] str.[h], C=C ring str.[h]), 981 (oop[i], -CH bend.[j] vibration of alkene), 758 (1,2 disubstitution)	($CDCl_3$, 400 MHz) δ 7.10 (d, *J* = 16 Hz, IH), 7.32-7.36 (m, 1H), 7.40-7.45 (ovlp. m, 6H), 7.55-7.58 (m, 2H)	($CDCl_3$, 100 MHz) δ 194.6, 146.6, 141.1, 134.3, 133.4, 131.4, 130.9, 129.1, 129.0, 128.6, 127.3, 126.1, 119.5	ESI MS [*m/z*, relative abundance] [287 $(M+H)^+$, 100], [309 $(M+Na)^+$, 10]
4e	3444, 3325 (-NH str.[h], primary amine), 1641 (α,β-unsaturated keto group, -C=O str.[h]), 1573 (-NH bend.[j], scissoring), 1336 (arom.[g] amino group, C-N str.[h]), 738 (1,2 disubstitution)	$CDCl_3$, 400 MHz) δ 7.91 (d, *J* = 4.8 Hz, 1H), 7.78 (dd, *J* = 4, 15.6 Hz, 1H), 7.63-7.68 (m, 3H), 7.44-7.45 (m, 3H), 7.31 (dd, *J* = 8, 16.8 Hz, 2H), 6.77 (app. d, *J* = 7.6 Hz, 2H)	($CDCl_3$, 100 MHz) δ 191.8, 165.5, 151.0, 143.0, 135.3, 134.3, 133.7, 131.0, 129.7, 128.9, 128.3, 123.2, 119.1, 117.3, 115.9	ESI MS [*m/z* relative abundance] [224 $(M+H)^+$,100], [199 $(C_8H_8)^+$, 42]
4f	3088 (arom.[g] -CH str.[h]), 1660 (α,β-unsaturated keto group, -C=O str.[h]), 1610 (asym.[k] str.[h] in $-NO_2$ group), 1527, 1344 (arom.[g] $-NO_2$ str.[h]), 870 (str.[h] of pi bond of N-O linkage), 763-790 ($-NO_2$ bend.[j] vibration)	($CDCl_3$, 500 MHz) δ 8.84 (dd, *J* = 2, 3.5 Hz, 1H), 8.45 (d, *J* = 8 Hz, 1H), 8.35 (d, *J* = 7.5 Hz, 1H), 7.90 (d, *J* = 15.5 Hz, 1H), 7.67-7.74 (m, 3H), 7.53 (d, *J* = 15.5 Hz, 1H), 7.44-7.46 (m, 2H)	($CDCl_3$, 125 MHz) δ 188.0, 148.5, 146.8, 139.5, 134.3, 134.1, 131.2, 130.0, 129.1, 128.8, 127.1, 123.3, 120.7	APCI MS [*m/z*, relative abundance] [254 $(M+H)^+$, 100], [208 $(C_{15}H_{11}O)^+$, 13], [149 $(C_{12}H_4)^+$, 8]
4g	3061 (arom.[g] -CH str.[h]), 1660 (α,β-unsaturated keto group, -C=O str.[h]), 1602 (=CH arom.[g] str.[h], C=C ring str.[h]), 796 (1,3 disubstitution), 758 (arom.[g] bend.[j])	($CDCl_3$, 500 MHz) δ 8.14 (dd, *J* = 1.5, 3.5 Hz, 1H), 7.93 (d, *J* = 7.5 Hz, 1H), 7.82 (d, *J* = 15.5 Hz, 1H), 7.71 (d, *J* = 7.5 Hz, 1H), 7.63-7.67 (m, 2H), 7.42-7.44 (m, 4H), 7.38 (dd, *J* = 8, 16 Hz, 1H)	($CDCl_3$, 125 MHz) δ 189.1, 145.7, 140.0, 135.6, 134.6, 131.5, 130.8, 130.2, 129.0, 128.6, 127.0, 123.0, 121.5	APCI MS [*m/z*, relative abundance] [289 $(M+2)^+$, 42], [287 $(M+H)^+$, 38], [208 $(287-Br)^+$, 100], [131 $(C_9H_6O)^+$, 28]
4h	3059 (arom.[g] -CH str.[h]), 2974, 2955 (aliphatic -CH str.[h]), 1653 (α,β-unsaturated keto group, -C=O str.[h]), 1600 (=CH- arom.[g] bend.[j]), 1332 (C-O-C str.[h]), 1224 (asym.[k] C-O-C str.[h]), 1182 (sym.[l] C-O-C str.[h]), 760 (oop[i] -CH bend.[j])	($CDCl_3$, 400 MHz) δ 8.05 (d, *J* = 8.4 Hz, 2H), 7.81 (d, *J* = 15.6 Hz, 1H), 7.65 (dd, *J* = 7.6, 9.6 Hz, 2H), 7.55 (d, *J* = 15.6 Hz, 1H), 7.41-7.44 (m, 3H), 6.99 (d, *J* = 8.8 Hz, 2H), 3.89 (s, 3H)	($CDCl_3$, 100 MHz) δ 188.8, 163.5, 144.0, 135.1, 131.1, 130.8, 130.3, 128.9, 128.4, 122.0, 113.9, 55.5	APCI MS [*m/z*, relative abundance] [239 $(M+H)^+$, 100], [221 $(239-H_2O)^+$, 5], [135 $(C_{11}H_3)^+$, 18]
4i	3053 (arom.[g] -CH str.[h]), 1656 (α,β-unsaturated keto group, -C=O str.[h]), 1597, 1444 (C=C ring str.[h]), 748 (arom.[g] bend.[j]), 860-800 (1,4 disubstitution)	($CDCl_3$, 500 MHz) δ 8.11 (d, *J* = 8.5 Hz, 2H), 7.85 (d, *J* = 15.5 Hz, 1H), 7.73 (d, *J* = 8 Hz, 2H), 7.64-7.67 (m, 4H), 7.58 (d, *J* = 15.5 Hz, 1H), 7.48 (t, *J* = 7.8 Hz, 2H), 7.38-7.44 (m, 4H)	($CDCl_3$, 125 MHz) δ 190.0, 145.6, 144.8, 140.0, 137.0, 135.0, 130.5, 129.1, 129.0, 128.5, 128.2, 127.3, 122.1	ESI MS [*m/z*, relative abundance] [285 $(M+H)^+$, 100], [307 $(M+Na)^+$, 10]

continude

Table 5: continude

Table 5: Spectral Characterization Data of Synthesized Compounds

4j	3055 (arom.[g] -CH str.[h]), 1656 (α,β-unsaturated keto group, -C=O str.[h]), 1602 (=CH arom.[g] str.[h], C=C ring str.[h]), 981 (oop[i], -CH bend.[j] vibration of alkene), 825 (1,4 disubstitution), 759 (arom.[g] bend.[j])	($CDCl_3$, 400 MHz) δ 7.81 (d, *J* = 8.4 Hz, 2H), 7.74 (d, *J* = 15.6 Hz, 1H), 7.56 (dd, *J* = 8.4, 14.4 Hz, 4H), 7.40 (d, *J* = 15.6 Hz, 1H), 7.34-7.41 (m, 3H)	($CDCl_3$, 100 MHz) δ 189.4, 145.4, 136.9, 134.7, 131.9, 130.8, 130.0, 129.0, 128.5, 127.9, 121.5	APCI MS [*m/z*, relative abundance] [289 $(M+2)^+$, 100], [287 $(M+H)^+$, 77], [207 $(M-Br)^+$, 25], [131 $(C_9H_7O)^+$, 24]

[g] = *arom. stands for aromatic;* [h] = *str. stands for stretch;* [i] = *oop stands for out-of-plane;* [j] = *bend. stands for bending;* [k] = *asym. stands for asymmetric;* [l] = *sym. stands for symmetric*

is added. Each experiment was performed in triplicate and average recorded.

RESULTS AND DISCUSSION

The data in Table No. 2 strongly suggests that all our ligands are drug-like and would be amenable to oral administration in humans. Moreover, TPSA of the target compounds was below 70 Å² implying easy permeability through cell membrane and ready penetrability across the BBB. **4i** is the only outlier in this dataset whose ADME LogP value of 5.93 is greater than the acceptable maximum of 5 and also falls outside the optimal range of –0.4 to +5.6 as suggested in later extensions. However, it is important to realize that this is only a rule of thumb which allows one violation per compound and is characterized by a number of exceptions to the rule especially in the therapeutic classes of vitamins, antibiotics and anti-fungals.

The ADMETox data presented in Table No. 3 suggest that all the compounds can cross BBB to the same or to a greater extent than ibuprofen which has somewhat reasonable permeability. Additionally, all the target ligands have a very good level of HIA after oral administration. Most of the chalcones except **4b** and **4e** have medium aqueous solubility. Interestingly, the aqueous solubility of these two compounds is comparable to that of ibuprofen suggesting that, if found active, they can be made druggable. All members of our designed library are likely to cause dose-dependent liver toxicity. This parameter, however, cannot be meaningfully interpreted before any animal studies are conducted to ascertain the dose required to elicit hepatotoxicity. With the exception of **4a** and **4b**, the rest of the compounds inhibit CYP2D6, a crucial CYP450 isoform which metabolizes nearly 20% of all drugs. A recent study which has primarily implicated CYP1A subfamily in the oxidative metabolism of chalcones,[20] therefore, points to the fact that the plasma levels of none of our target compounds will fluctuate because of their inherent CYP2D6-inhibitory potential. The high PPB (≥ 90%) exhibited by all the chalcones will likely impart a longer duration of action. Besides, this attribute clearly underlines their lipophilicity that was also evident from their excellent HIA.

After the preliminary *in silico* filters indicated that the compounds, in fact, could be viable lead candidates, the target chalcones **4a-j** were synthesized according to one of the three well-established methods under conditions depicted in Fig. 2. All methods involve a single step and are procedural variations on a classic base-catalyzed aldol condensation reaction of appropriately substituted acetophenones **2a-j** with benzaldehyde **3**. While methods A and B are conventional solution phase syntheses, method C involves a microwave-assisted preparation. Each of the three methods were attempted for preparing the title compounds and only the method giving maximal, reproducible yields reported herein. The physical properties of all the compounds are summarized in Table No. 4 while their structures were confirmed by relevant spectral data that is presented in Table No. 5.

Denaturation of proteins is a well documented cause of inflammation. Hence, an *in vitro* assay based on the capability of the test compounds to inhibit the process of protein denaturation was employed to assess the anti-inflammatory activity of the chalcone derivatives synthesized. The findings illustrated in Fig. 3 indicated a concentration-dependent inhibition of protein denaturation by the chalcone library throughout the concentration range of 10 to 30 μg/ml.

Moreover, the target chalcones exhibited a stronger inhibitory potential compared to that of ibuprofen which also exhibited an analogous increase in inhibition of protein denaturation over the same concentration range. Upon closer inspection of the data, it is clear that substitution pattern on the phenyl ring of the acetophenic group of chalcone moiety plays an important role in containing inflammation. Among the synthesized chalcones, compound **4b** was the most potent; compounds **4e**, **4g**, **4h** and **4j** showed better activity in comparison with the standard drug ibuprofen, while compounds **4c**, **4d**, **4f** and **4i** exhibited activity similar to that of the reference drug.

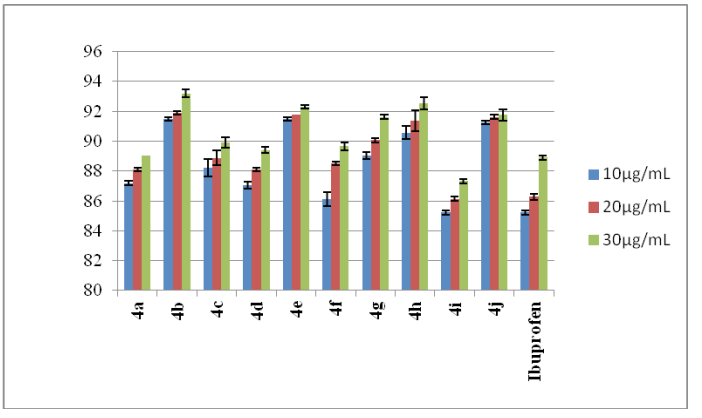


Figure 3: *In vitro* anti-inflammatory activity.

In this series, small hydrophilic +*M* groups (like hydroxy in **4b**, amino in **4e** and methoxy in **4h**) seem to be favorable for activity irrespective of their point of attachment in the ring. The inductively electron-withdrawing bromo group that was appended to ring-A forming all three regioisomeric monosubstituted bromochalcones showed a preference for the 4'- (*p*-) position over 3'- (*m*-) position. This is clear from the superior anti-inflammatory potential of **4j** over **4g**. Placing a -*I* group at the 2'- (*o*-) position does not seem to alter the activity as is evident from the equivalent inhibitory potential of compound **4d** and the unsubstituted chalcone **4a**. Presence of a +*I* group (like methyl in **4c**) or a -M group (like nitro in **4f**) seems to have no effect in the activity of the parent chalcone **4a**. The suboptimal anti-inflammatory potential of compound **4i** suggested that bulky, hydrophobic groups may be unsuitable as substituents. It is interesting to note in this context that the 4'-phenylchalcone **4i** also presented a violation with regard to its ADME LogP in the drug-likeness assessment.

CONCLUSION

The synthesis and preliminary biological evaluation of a focused library of ring-A monosubstituted chalcones was carried out in an effort to shed some light on the structural requirements of the acetophenic phenyl group in the moiety for eliciting anti-inflammatory activity. *In silico* drug-likeness and druggability assessments indicated that most of the ligands were suitable for development as oral candidates. Biological evaluation of the synthesized compounds showed that a single substituent in ring-A is not detrimental to the activity of the chalcone scaffold. Preliminary data pointed to a preference for small, hydrophilic +*M* groups in ring-A with compound **4b** emerging as a promising lead. The synthesis and evaluation of focused libraries of similar nature is ongoing to elucidate precise correlations between structure and anti-inflammatory activity. We hope that such a rational medicinal chemistry approach applied to mini series of small molecules will pave the way for clear elucidation of structure function correlations.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. G K Suraishkumar, Head-SAIF at IIT Madras and Dr. Jacob Philip, Director-STIC at CUSAT Kochi for providing the facilities to record NMR and mass spectra. We are also grateful to Mr. N Srinivasan, Director-Porus Labs Pvt. Ltd., Hyderabad for providing a gift sample of ibuprofen as well as to Mr. Rahul R of Amrita School of Pharmacy for his help in conducting the anti-inflammatory testing on the synthesized compounds.

REFERENCES

1. Dhar DN. The chemistry of chalcones and related compounds. 1st ed. New York: Wiley; 1981.
2. Kontogiorgis C, Mantzanidou M, Hadjipavlou-Litina D. Chalcones and their potential in inflammation. Mini Rev Med Chem 2008; 8(12):1224–42.
3. Nowakowska Z. A review of anti-infective and anti-inflammatory chalcones. Eur J Med Chem 2007; 42(2):125–37.
4. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents 2005; 26(5):343–56.
5. Batovska DI, Todorova IT. Trends in utilization of the pharmacological potential of chalcones. Curr Clin Pharmacol 2010; 5(1):1–29.
6. Mojzis J, Varinska L, Mojzisova G, Kostova I, Mirossay L. Antiangiogenic effects of flavonoids and chalcones. Pharmacol Res 2008; 57(4):259–65.
7. Kidd BL, Urban LA. Mechanisms of inflammatory pain. Br J Anaesth 2001; 87(1):3–11.
8. Chiaradia LD, Dos Santos R, Vitor CE *et al.* Synthesis and pharmacological activity of chalcones derived from 2,4,6-trimethoxy acetophenone in RAW 264.7 cells stimulated by LPS: quantitative structure-activity relationships. Bioorg Med Chem 2008; 16(2):658–67.
9. Pandey MK, Sandur SK, Sung B, Sethi G, Kunnumakkara AB, Aggarwal BB. Butein, a tetrahydroxychalcone, inhibits nuclear factor (NF)-kappaB and NF-kappaB-regulated gene expression through direct inhibition of IkappaBalpha kinase beta on cysteine 179 residue. J Biol Chem 2007; 282(24):17340–50.

10. Folmer F, Blasius R, Morceau F *et al*. Inhibition of TNFalpha-induced activation of nuclear factor kappaB by kava (Piper methysticum) derivatives. Biochem Pharmacol 2006; 71(8):1206–18.
11. Alcaraz MJ, Vicente AM, Araico A, Dominquez JN, Terencio MC, Ferrandiz ML. Role of nuclear factor-kappaB and heme oxygenase-1 in the mechanism of action of an anti-inflammatory chalcone derivative in RAW 264.7 cells. Br J Pharmacol 2004; 142(7):1191–9.
12. Sawle P, Moulton BE, Jarzykowska M *et al*. Structure-activity relationships of methoxychalcones as inducers of heme oxygenase-1. Chem Res Toxicol 2008; 21(7):1484–94.
13. Jin F, Jin XY, Jin YL *et al*. Structural requirements of 2',4',6'-tris (methoxymethoxy) chalcone derivatives for anti-inflammatory activity: the importance of a 2'-hydroxy moiety. Arch Pharm Res 2007; 30(11): 1359–67.
14. Foresti R, Hoque M, Monti D, Green CJ, Motterlini RJ. Differential activation of heme oxygenase-1 by chalcones and rosolic acid in endothelial cells. Pharmacol Exp Ther 2005; 312(2):686–93.
15. Zarghi A, Zebardast T, Hakimion F, Shirazi FH, Rao PNP, Knaus EE. Synthesis and biological evaluation of 1,3-diphenylprop-2-en-1-ones possessing a methanesulfonamido or an azido pharmacophore as cyclooxygenase-1/-2 inhibitors. Bioorg Med Chem 2006; 14(20):7044–50.
16. Balasubramanian R, Vijayagopal R. Design and *in silico* analysis of ring-A monosubstituted chalcones as potential anti-inflammatory agents. Bull Pharm Res 2012; 2(2):70–7.
17. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Del Rev 1997; 23(1–3):3–25.
18. Ghose AK, Viswanadhan VN, Wendoloski JJ. A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. J Comb Chem 1999; 1(1):55–68.
19. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and anti inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J Pharm Pharm Sci 2010; 2(1):146–55.
20. Kohno Y, Kitamura S, Sanoh S, Sugihara K, Fujimoto N, Ohta S. Metabolism of the α,β-unsaturated ketones, chalcone and *trans*-4-phenyl-3-buten-2-one, by rat liver microsomes and estrogenic activity of the metabolites. Drug Metab Disp 2005; 33(8):1115–23.