Full Paper

Arylalkyl Ketones, Benzophenones, Desoxybenzoins and Chalcones Inhibit TNF-α Induced Expression of ICAM-1: Structure-Activity Analysis

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The interaction between leukocytes and the vascular endothelial cells (EC) via cellular adhesion molecules plays an important role in the pathogenesis of various inflammatory and autoimmune diseases. Small molecules that block these interactions have been targeted as potential therapeutic agents against acute and chronic inflammatory diseases. In an effort to identify potent intercellular cell adhesion molecule-1 (ICAM-1) inhibitors, a large number of arylalkyl ketones, benzophenones, desoxybenzoins and chalcones and their analogs (54 in total) have been synthesized and screened for their ICAM-1 inhibitory activity. The structure-activity relationship studies of these compounds identified three potent chalcone derivatives and also demonstrated the possible mechanism for their ICAM-1 inhibitory activities. The most active compound was found to be **79**.

Keywords: Arylalkyl ketones / Benzophenones / Chalcones / Desoxybenzoins / Endothelial cells / ICAM-1

Received: August 3, 2011; Revised: October 17, 2011; Accepted: October 21, 2011

DOI 10.1002/ardp.201100279

Introduction

The recruitment of leukocytes from the blood into tissue is a multistep process that requires a series of leukocyte-endothelial adhesive interactions, involving several families of adhesion molecules. They participate in inflammatory reactions mainly by regulation of leukocyte migration, activation

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Abbreviations: ICAM-1, intercellular adhesion molecule-1; **VCAM-1,** vascular cell adhesion molecule-1; **TNF-α,** tumor necrosis factor α; **NF-κB,** nuclear factor κB; **ECs,** endothelial cells; **Nrf2**, nuclear factor erythroid-2 related factor 2.

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and survival. Elevated expression of the cell adhesion molecules such as ICAM-1, VCAM-1, and E-selectin on the luminal surface of vascular endothelial cells is a critical event in inflammatory processes. Small molecules that block these interactions have been targeted for potential therapeutic treatments of acute and chronic inflammatory diseases. Anti-adhesion therapy by using specific monoclonal antibodies (mAbs) has been found to be beneficial for controlling various diseases [1, 2]. However, due to endotoxin contamination, unpredictable secondary antibody formation, cellular activation, and other complications like serum sickness and anaphylaxis, the practical use of mAbs is limited. However, inhibition of expression of ICAM-1, VCAM-1 and E-selectin by various small molecules from natural and synthetic sources such as curcumin, glucocorticoids, pentoxifylline, etc. have been shown to downregulate the expression of cell adhesion molecules and are effective in controlling various inflammatory diseases [3–5].

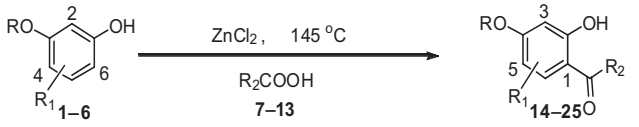
Arylalkyl ketones, benzophenones, desoxybenzoins and chalcones are groups of compounds widely present in the higher plants and have been synthesized in the laboratory [6, 7]. They contain an aromatic ring and an alkyl moiety or two aromatic rings separated by a 1–3 carbon chain (saturated or unsaturated). This unique structural feature is responsible for various activities of these molecules. Chalcones have been reported to possess a wide variety of biological properties including anti-inflammatory, analgesic and antipyretic [8–10], antioxidant [11], antibacterial, antifungal and antiprotozoal activities [12–14]. They are also reported to be gastric protectant, anti-mutagenic and antitumorogenic [10]. Substitution pattern on chalcone rings plays a critical role in its biological properties. Various substituents on the chalcone rings either enhance or decrease the biological activity. We have reported the cell adhesion molecules inhibitory activity of the natural chalcone, isoliquiritigenin [15]. In this study, we are reporting the synthesis and ICAM-1 inhibitory activities of four similar classes of compounds (arylalkyl ketones, benzophenones, desoxybenzoins and chalcones and their analogs, 54 in total) to draw a structure-activity relationship; a possible mechanism for this activity is also proposed.

Results and discussion

Arylalkyl ketones inhibit the TNF-α induced expression of ICAM-1 on endothelial cells

The arylalkyl ketones **14–25** were synthesized by the Friedel–Crafts acylation of the corresponding polyphenols **1–6** with different aliphatic acids **7–13** (Scheme 1) and evaluated for their effect on the inhibition of TNF-α induced expression of ICAM-1 on endothelial cells. The % inhibition and IC_{50} values of the screened ketones are summarized in Table 1. 1-(2,4,6-Trihydroxyphenyl)-ethanone (**21**) was found to be the most potent inhibitor of TNF-α induced expression of ICAM-1 with IC_{50} of 178 μM and 70% of ICAM-1 inhibition, whereas its dihydroxy analog **14** possesses IC_{50} of 328 μM and 70% of ICAM-1 inhibition (Table 1). However, its analogs having longer alkyl chains, i.e. *n*-butyl, *iso*-pentyl, hexyl, nonyl and pentadecyl moieties, possess relatively lower % inhibition values (Table 1).

This clearly illustrates the significance of a greater number of free phenolic hydroxyl groups on the benzene ring and the length of the alkyl group present in the aryl-alkyl ketones. When the number of free phenolic hydroxyl groups is higher, the inhibition values are relatively higher, whereas a longer alkyl chain lowers the inhibition values (Table 1). Moreover %



Comp.	R	R_1
1	H	H
2	H	5-OH
3	CH_3	4-OH
4	H	2,5-$(OCH_3)_2$
5	CH_3	H
6	CH_3	5-OCH_3

Acid	R_2
7	CH_3
8	C_2H_5
9	*n*-C_4H_9
10	*iso*-C_5H_{11}
11	*n*-C_6H_{13}
12	*n*-C_9H_{19}
13	*n*-$C_{15}H_{31}$

Comp.	R	R_1	R_2
14	H	H	CH_3
15	H	H	C_2H_5
16	H	H	*n*-C_4H_9
17	H	H	*iso*-C_5H_{11}
18	H	H	*n*-C_6H_{13}
19	H	H	*n*-C_9H_{19}
20	H	H	*n*-$C_{15}H_{31}$
21	H	6-OH	CH_3
22	H	5-OH	CH_3
23	CH_3	3,6-$(OCH_3)_2$	C_2H_5
24	CH_3	H	CH_3
25	CH_3	6-OCH_3	CH_3

Scheme 1. Synthesis of different arylalkyl ketones.

Table 1. Effect of arylalkyl ketones on the TNF-α induced expression of ICAM-1 on endothelial cells.

Compound	% Inhibition	Concentration μM*	IC_{50} μM
1-(2,4-Dihydroxyphenyl)-ethanone (**14**)	70	460	328
1-(2,4-Dihydroxyphenyl)-propan-1-one (**15**)	40	421	–
1-(2,4-Dihydroxyphenyl)-pentan-1-one (**16**)	35	308	–
1-(2,4-Dihydroxyphenyl)-4-methylpentan-1-one (**17**)	40	240	–
1-(2,4-Dihydroxyphenyl)-heptan-1-one (**18**)	30	224	–
1-(2,4-Dihydroxyphenyl)-decan-1-one (**19**)	25	151	–
1-(2,4-Dihydroxyphenyl)-hexadecan-1-one (**20**)	10	114	–
1-(2,4,6-Trihydroxyphenyl)-ethanone (**21**)	70	237	178
1-(2,5-Dihydroxy-4-methoxyphenyl)-ethanone (**22**)	55	219	197
1-(2,4-Dihydroxy-3,6-dimethoxyphenyl)-propan-1-one (**23**)	50	221	221
1-(2-Hydroxy-4-methoxyphenyl)-ethanone (**24**)	55	300	282
1-(2-Hydroxy-4,6-dimethoxyphenyl)-ethanone (**25**)	40	356	–

* The concentration levels of different compounds are based on their maximum tolerable concentration by the cells. The data presented are representative of three independent experiments. Values shown are means ± SD of quadruplicate wells.

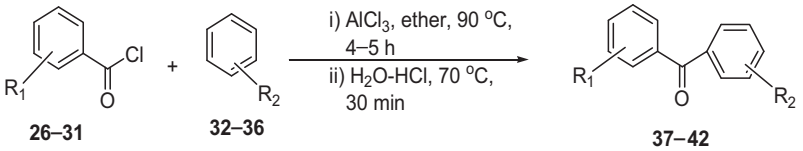
inhibition of ICAM-1 expression was found to decrease as the number of methoxy groups in the aromatic moiety increases. Thus compound **21** lacking any methoxy group shows the maximum inhibition value of 70% (Table 1); following the trend, the compounds **22** and **24** (each showing 55% inhibition and having one methoxy group) and the compounds **23** and **25** (both having two methoxy groups and two and one hydroxyl groups, respectively) show 50% and 40% inhibition of ICAM-1 expression activity; the same trend is followed in terms of the IC_{50} values of these compounds (Table 1).

Benzophenones inhibit the TNF-α induced expression of ICAM-1 on endothelial cells

Benzophenones **37–42** were synthesized by the Friedel-Crafts acylation of various derivatives of benzene with different benzoyl chloride analogs (Scheme 2) and evaluated for their effect on the inhibition of TNF-α induced expression of ICAM-1 (Table 2). 2,4-Dihydroxyphenyl, phenylmethanone (**37**) was found to be the most active among the screened benzophenones with IC_{50} of 256 μM and 85% inhibition of ICAM-1 expression, followed by the compounds 2,6-dihydroxyphenyl, phenylmethanone (**40**) and 2-carboxyphenyl, 2-hydroxy-4-methylphenylmethanone (**42**) (exhibiting 55% ICAM-1 inhibition, IC_{50} 326 μM and 50% ICAM-1 inhibition, IC_{50} 351 μM values, respectively). All of these three compounds possess two polar groups, either two phenolic hydroxyl groups or one hydroxyl and a carboxy group at the conjugated positions to the carbonyl group. Remaining compounds of this class *viz*. **38**, **39** and **41** had insignificant activity in lowering the ICAM-1 expression as they possess 1–4 methoxy groups. Thus, like arylalkyl ketones, benzophenones possessing more free hydroxyl groups showed better activity and compounds having more methoxy groups showed low activity.

Desoxybenzoins inhibit the TNF-α induced expression of ICAM-1 on endothelial cells

The desoxybenzoins **55–64** having an additional methylene group between the two aromatic rings, as compared to benzophenones were synthesized (Scheme 3) and screened for their effect on the TNF-α induced expression of ICAM-1.



26–31 **32–36** **37–42**

Compd.	R_1
26	2,4-$(OH)_2$
27	2-OH, 5-OCH_3
28	2,5 $(OH)_2$, 4-OCH_3
29	H
30	2-COOH, 6-NH_2
31	2-COOH

Compd.	R_2
32	H
33	1,3,5-$(OCH_3)_3$
34	1,5-$(OH)_2$
35	1-OCH_3, 3-CH_3
36	1-OH, 3-CH_3

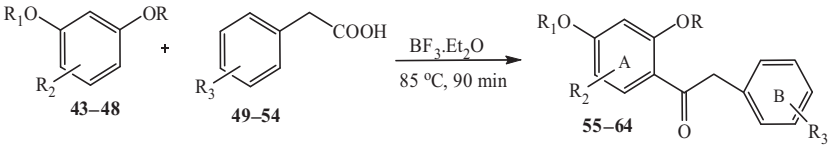
Compd.	R_1	R_2
37	2,4-$(OH)_2$	H
38	2-OH, 5-OCH_3	2,4,6-$(OCH_3)_3$
39	2,5-$(OH)_2$, 4-OCH_3	H
40	H	2,6-$(OH)_2$
41	2-COOH, 6-NH_2	2-OCH_3, 4-CH_3
42	2-COOH	2-OH, 4-CH_3

Scheme 2. Synthesis of different benzophenones.

Table 2. Effect of benzophenones on the TNF-α induced expression of ICAM-1 on endothelial cells.

Compound	% Inhibition	Concentration μM*	IC_{50} μM
2,4-Dihydroxyphenyl, phenylmethanone (**37**)	85	326	256
2-Hydroxy-5-methoxyphenyl,2,4,6-trimethoxyphenylmethanone (**38**)	45	251	–
2,5-Dihydroxy-4-methoxyphenyl, phenylmethanone (**39**)	35	327	–
2,6-Dihydroxyphenyl, phenylmethanone (**40**)	55	373	326
2-Carboxy-6-aminophenyl,2-methoxy-4-methylphenylmethanone (**41**)	35	350	–
2-Carboxyphenyl,2-hydroxy-4-methylphenylmethanone (**42**)	50	351	351

* The concentration levels of different compounds are based on their maximum tolerable concentration by the cells. The data presented are representative of three independent experiments. Values shown are means ± SD of quadruplicate wells.



Compd.	R	R_1	R_2
43	H	H	5-OH
44	H	H	5-CH_3
45	H	CH_3	2-OCH_3
46	Ac	Ac	H
47	H	CH_3	H
48	H	H	H

Compd.	R_3
49	3,4-$(OCH_3)_2$
50	H
51	4-OCH_3
52	4-OH
53	2,4-$(OCH_3)_2$
54	3,4 -OCH_2O-

Compd.	R	R_1	R_2	R_3
55	H	H	6-OH	3,4-$(OCH_3)_2$
56	H	H	6-CH_3	H
57	H	CH_3	3-OCH_3	H
58	H	H	6-CH_3	4-OCH_3
59	H	H	6-OH	H
60	H	H	6-OH	4-OH
61	H	H	6-OH	2,4-$(OCH_3)_2$
62	Ac	Ac	H	H
63	H	CH_3	H	3,4 -OCH_2O-
64	H	H	H	4-OCH_3

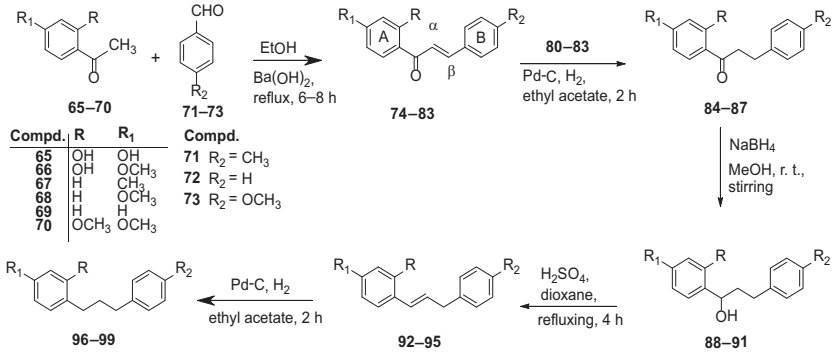
Scheme 3. Synthesis of different desoxybenzoins.

Table 3 summarizes the results of this screening; 1-(2-hydroxy-4-methoxyphenyl)-2-(3,4-methylenedioxyphenyl)-ethanone (**63**) was found to be the most active compound (Table 3) with IC_{50} of 139 μM and 85% of ICAM-1 inhibition. 2-Phenyl-1-(2,4,6-trihydroxyphenyl)-ethanone (**59**) was also comparable in its activity to that of **63** with IC_{50} of 184 μM and 80% of ICAM-1 inhibition. Again desoxybenzoins having greater number of free phenolic hydroxyl groups in the aromatic ring 'A' (conjugated to the carbonyl group) show better ICAM-1 expression inhibition values, e.g. compounds **56**, **59**, **60**, **62**, **63** and **64**, all have one to three free phenolic hydroxyl groups at the conjugated positions to the carbonyl group and showed inhibition values above 60% (Table 3). The compounds **55**, **57**, **58** and **61**, though containing two or three free phenolic groups in the ring 'A', they also possess two methoxy groups; like aryl alkyl ketones and benzophenones,

Table 3. Effect of desoxybenzoins on the TNF-α induced expression of ICAM-1 on endothelial cells.

Compound	% Inhibition	Concentration μM*	IC_{50} μM
2-(3,4-Dimethoxyphenyl)-1-(2,4,6-trihydroxyphenyl)-ethanone (**55**)	50	328	328
1-(2,4-Dihydroxy-6-methylphenyl)-2-phenylethanone (**56**)	60	371	330
1-(2-Hydroxy-3,4-dimethoxyphenyl)-2-phenylethanone (**57**)	50	367	367
1-(2,4-Dihydroxy-6-methylphenyl)-2-(4-methoxyphenyl)-ethanone (**58**)	30	367	–
2-Phenyl-1-(2,4,6-trihydroxyphenyl)-ethanone (**59**)	80	245	184
2-(4-Hydroxyphenyl)-1-(2,4,6-trihydroxy-phenyl)-ethanone (**60**)	60	384	326
2-(2,4-Dimethoxyphenyl)-1-(2,4,6-trihydroxyphenyl)-ethanone (**61**)	40	295	–
1-(2,4-Diacetoxyphenyl)-2-phenylethanone (**62**)	65	256	224
1-(2-Hydroxy-4-methoxyphenyl)-2-(3,4-methylenedioxyphenyl)-ethanone (**63**)	85	209	139
1-(2,4-Dihydroxyphenyl)-2-(4-methoxyphenyl)-ethanone (**64**)	65	271	232

* The concentration levels of different compounds are based on their maximum tolerable concentration by the cells. The data presented are representative of three independent experiments. Values shown are means ± SD of quadruplicate wells.



Compd.	R	R_1
65	OH	OH
66	OH	OCH_3
67	H	CH_3
68	H	OCH_3
69	H	H
70	OCH_3	OCH_3

Compd.
71 $R_2 = CH_3$
72 R_2 = H
73 $R_2 = OCH_3$

Compound	R	R_1	R_2
74	OH	OH	CH_3
75	OH	OCH_3	H
76	H	CH_3	OCH_3
77	OH	OH	H
78	H	CH_3	H
79	OCH_3	OCH_3	H
80, 84, 88, 92 and **96**	H	OCH_3	OCH_3
81, 85, 89, 93 and **97**	H	H	H
82, 86, 90, 94 and **98**	H	H	OCH_3
83, 87, 91, 95 and **99**	H	OCH_3	H

Scheme 4. Synthesis of different chalcones.

desoxybenzoins carrying methoxy groups also showed lower ICAM-1 expression inhibition values.

The compound **62** contains two acetoxy groups in ring 'A', as per earlier report [16], the acetoxy groups under test conditions get deacetylated to furnish the corresponding dihydroxy compound *in situ* having two free phenolic hydroxy groups at the conjugated positions. Interestingly, the most active compound of the series, i.e. **63**, possesses the methylenedioxyphenyl moiety, and we are synthesizing more analogs possessing this moiety to establish the effect of the methylenedioxy group and possible mechanism of their action. The structure-activity analysis of arylalkyl ketones, benzophenones and desoxybenzoins (possessing one or two carbon atom chain between the aromatic rings, thus lacking extended conjugation between the aromatic rings) has revealed that compounds of all the three classes possessing more free hydroxyl groups showed higher activity and compounds having more methoxy groups showed lower ICAM-1 expression inhibition activity.

Chalcones inhibit the TNF-α induced expression of ICAM-1 on endothelial cells

When we introduced three carbons in between the two aromatic rings (i.e. in chalcones), the ICAM-1 inhibitory activity of these compounds was the highest as compared to those of arylalkyl ketones and benzophenones (having one carbon in between the two aromatic rings) and desoxybenzoins (having two carbon atoms between the two aromatic rings). For clear structure-activity relationship among chalcones, we have synthesized a series of ten chalcone derivatives, *viz.* **74**–**83**, via aldol condensation between various benzaldehydes and different acetophenones in the presence of barium hydroxide as catalyst and their analogs having different structural features in the three-carbon moiety separating the two aromatic rings (Scheme 4). These derivatives were evaluated for their ICAM-1 inhibitory activity; Table 4 summarizes the results of this activity. Interestingly, most of the screened chalcones were active except for the compounds **76**, **80** and **82** (Table 4). Three chalcones, *viz.* **75**, **77**and **79** (all having the unsubstitued C-3 phenyl moiety and the 2,4-dioxygenated C-1 phenyl moiety), showed highest ICAM-1 inhibition (each exhibited 90% inhibition, Table 4).

However, the IC_{50} values of these three chalcones decrease (hence ICAM-1 expression inhibition capacity increases) as we protect one of the two hydroxyl groups in the C-1 phenyl moiety in compound **77** (IC_{50} 166 μM, Table 4) to get its monoether, *viz.* this compound **75** (IC_{50} 58 μM, Table 4), and when we further look at the diether of **77**, i.e. compound **79** (IC_{50} 37 μM, Table 4), the activity still increases. Thus, SAR studies on chalcones indicate that the most active compounds of this series in inhibiting the ICAM-1 expression should have the C-2, C-4 dioxygenation pattern in the C-1 phenyl ring (preferably both substituents as ether moieties) and no substituent in the C-3 phenyl moiety. Interestingly it

Table 4. Effect of chalcones and their analogs/derivatives on the TNF-α induced expression of ICAM-1 on endothelial cells.

Compound	% Inhibition	Concentration* μM	IC_{50} μM
1-(2,4-Dihydroxyphenyl)-3-(4-methylphenyl) propenone (**74**)	60	39	29
1-(2-Hydroxy-4-methoxyphenyl)-3-phenylpropenone (**75**)	90	78	58
3-(4-Methoxyphenyl)-1-(4-methylphenyl) propenone (**76**)	40	39	–
1-(2,4-Dihydroxyphenyl)-3-phenylpropenone (**77**)	90	291	166
1-(4-Methylphenyl)-3-phenyl-propenone (**78**)	85	44	35
1-(2,4-Dimethoxyphenyl)-3-phenylpropenone (**79**)	90	58	37
1,3-Bis-(4-methoxyphenyl)-propenone (**80**)	8	149	–
1,3-Diphenylpropenone (**81**)	70	96	81
3-(4-Methoxyphenyl)-1-phenylpropenone (**82**)	34	124	–
1-(4-Methoxyphenyl)-3-phenylpropenone (**83**)	70	293	188
1,3-Bis-(4-methoxyphenyl)propanone (**84**)	5	332	–
1,3-Diphenylpropanone (**85**)	55	195	–
3-(4-Methoxyphenyl)-1-phenylpropanone (**86**)	10	374	–
1-(4-Methoxyphenyl)-3-phenylpropanone (**87**)	15	166	–
1,3-Bis-(4-methoxyphenyl)-propan-1-ol (**88**)	10	146	–
1,3-Diphenylpropan-1-ol (**89**)	20	329	–
3-(4-Methoxyphenyl)-1-phenylpropan-1-ol (**90**)	12	247	–
1-(4-Methoxyphenyl)-3-phenylpropan-1-ol (**91**)	15	247	–
1,3-Bis-(4-methoxyphenyl)propene (**92**)	31	353	–
1,3-Diphenylpropene (**93**)	40	308	–
3-(4-Methoxyphenyl)-1-phenylpropene (**94**)	35	401	–
1-(4-Methoxyphenyl)-3-phenylpropene (**95**)	40	312	–
1,3-Bis-(4-methoxyphenyl)propane (**96**)	15	195	–
1,3-Diphenylpropane (**97**)	35	254	–
3-(4-Methoxyphenyl)-1-phenylpropane (**98**)	15	220	–
1-(4-Methoxyphenyl)-3-phenylpropane (**99**)	18	214	–

* The concentration levels of different compounds are based on their maximum tolerable concentration by the cells. The data presented are representative of three independent experiments. Values shown are means ± SD of quadruplicate wells.

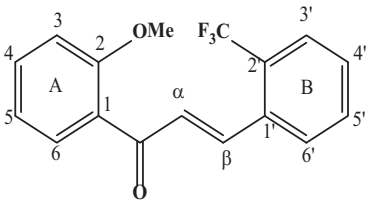
is quite opposite to the effects of free phenolic hydroxyl groups and ether moieties on the skeletons of arylalkyl ketones, benzophenones and desoxybenzoins.

To reveal the mechanism of action of chalcones and identify the pharmacophore, we wanted to see the effect of extended conjugation in chalcones due to the presence of the three-carbon atom α,β-unsaturated carbonyl moiety in chalcones by examining three different types of chalcone derivatives: (i) those lacking the C–C double bond, (ii) those lacking the carbonyl group and (iii) those lacking both the C–C double bond and the carbonyl group.

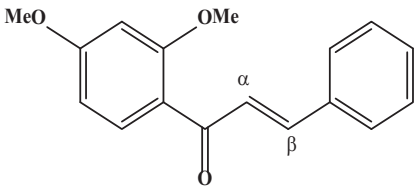
In order to evaluate the significance of the C–C double bond (unsaturation) and that of the carbonyl group present in the chalcone skeleton, we have synthesized a large number of analogs without the C-2, C-3 double bond, but still having the C-1 carbonyl group (compounds **84–87**) by reducing the C–C double bond of **80–83** (Scheme 4).

Activity evaluation of these compounds affirmed that the TNF-α induced ICAM-1 expression lowering activity of these compounds sharply dropped down (Table 4). This clearly signifies the importance of the C–C double bond in inhibiting the TNF-α induced ICAM-1 expression. Further to establish the importance of carbonyl group, we have carried out a series of reduction/dehydration reactions on the compounds **84–87** (Scheme 4) to obtain two groups of compounds: (i) having the C–C double bond but lacking the carbonyl group (i.e. compounds **92–95**) and (ii) compounds lacking both the carbonyl group and the C–C double bond (i.e. compounds **88–91** and **96–99**). However, both the groups of compounds lacking the carbonyl group, i.e. the hydroxy analogs, 1,3-diphenylpropanols **88–91** (prepared by reducing the carbonyl group of chalcones by $NaBH_4$) and the compounds **92–95** (1,3-diphenylpropenes prepared by dehydration of **88–91**), have shown lower activity compared to the analogs containing carbonyl group, i.e. compounds **84–87** (Table 4). Furthermore, fully saturated analogs, i.e. the 1,3-diphenylpropanes **96–99**, obtained from **92–95** by reducing their double bond, lack both the carbonyl group and C=C bond and were found to be least active (Table 4). This extensive study on different variants of chalcones proves that the presence of both the C–C double bond and the carbonyl group in the three-carbon moiety separating the two aromatic rings is important and could be responsible for the ICAM-1 expression lowering capacity of chalcones.

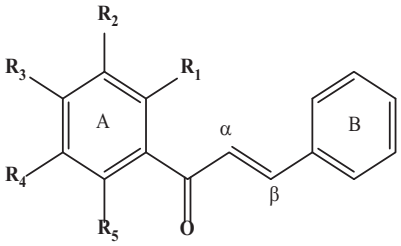
It is interesting to mention here that in our separate yet related study, we have synthesized a series of differently substituted chalcones and their analogs (54 compounds) having groups like methoxy, trifluoromethyl, nitro, etc. [16]. Most of



Most active, high solubility and non-toxic
Ref. No. 17, Kumar *et al.*



Most active, Compound 79 from our present study (Scheme 4, Table 4)



R_1, R_2, R_3, R_4, R_5 = H, OMe, CF_3, NO_2, CH_3

Figure 1. Structures of the most active compounds.

these chalcones have trifluoromethyl group in the ring B and one to three methoxy groups at the different positions in the ring A, and have determined their anti-inflammatory activities by a different test system, *viz*. modification results of cysteine residue in Keap 1, an inhibitor of anti-inflammatory transcription factor Nrf2, by a variety of inducers which can act as Michael acceptors (like chalcones). The binding of chalcones results in conformational changes that render Keap 1 to dissociate from Nrf2, thereby activating Nrf2 and potentiating its antiinflammatory activity in epithelial cells. Importantly, this study also showed the requirement of α,β- unsaturated carbonyl moiety for the chalcones to exhibit the antioxidant and anti-inflammatory activities [16]. We have also observed that when methoxy group is present at C-2 position in the ring A, they show greater activity which is consistant with our present study (Figure 1). Most of the chalcones having the C-2, C-4 dimethoxy substitution pattern exhibit greater activity than others. The most active compound of the present study is compound **79** (Scheme 4, Table 4). Further, on comparison of the activities of chalcones (Table 2, Figure 1, ref. 17) that contain two methoxy groups at the C-2, C-4 positions show relatively higher activities, which is the substitution pattern of the most active compound **79** of the present study (Figure 1).

Thus, in the present study, we have identified three chalcone derivatives, *viz.* compounds **75**, **77** and **79,** which inhibited TNF-α induced ICAM-1 expression on human endothelial cells with IC_{50} values of 58, 166, and 37 μM, respectively. As expression of cell adhesion molecules including ICAM-1 plays a crucial role in inflammatory diseases, inhibition of ICAM-1 has been suggested to be one of the most promising therapeutic modalities. In this context, compounds **75**, **77** and **79** having comparatively lower IC_{50} values than the commonly used anti-inflammatory drugs such as diclofenac (IC_{50} 750 μM), *N*-acetylcysteine (IC_{50} 100 μM), pyrrolidine dithiocarbamate (IC_{50} 1000 μM), etc. could be useful as lead molecules towards developing anti-inflammatory drugs in the future.

Conclusion

In conclusion, we have synthesized a large number of different analogs of naturally occurring arylalkyl ketones, benzophenones, desoxybenzoins and chalcones and evaluated their ICAM-1 expression inhibitory effect. Among all the four different classes of compounds screened in the present investigation, chalcones (which have the three-carbon atom α,β-enone chain between the two aromatic rings) show moderate to very high ICAM-1 expression inhibitory activity. The most active compound was found to be the chalcone **79**. Further modifications are under study in order to improve the activity and find a lead/hit towards clinical anti-inflammatory agent which would be reported in due course.

Experimental section

The organic solvents (acetone, ethyl acetate, methanol, chloroform, ethanol and petroleum ether) were dried and distilled prior to their use. Analytical TLCs were performed on precoated Merck silica gel 60 F_{254} plates; the spots were visualized under UV light. Melting points were determined in a sulphuric acid bath and are uncorrected. The IR spectra were recorded on a Perkin-Elmer model 2000 FT-IR spectrophotometer. The ^{1}H NMR

and ^{13}C NMR spectra were recorded on a Bruker Avance instrument at 300 MHz and 75.5 MHz, respectively using TMS as internal standard. The HRMS were recorded on a TMS-AX 505 W instrument. Anti-ICAM-1 antibody and TNF-α were purchased from Pharmingen, USA; M199, L-glutamine, penicillin, streptomycin, amphotericin, endothelial cell growth factor, trypsin, Pucks saline, HEPES, DMSO, *o*-phenylenediamine dihydrochloride and anti-mouse IgG-HRP were purchased from Sigma Chemical Co., USA. Fetal calf serum was purchased from Biological Industries, Israel. NADPH, ADP and trichloroacetic acid (TCA) were obtained from Sisco Research Laboratory (Mumbai, India).

Synthesis of aryl-alkyl ketones

Friedel-Crafts acylation

The heterogenous mixture of fused $ZnCl_2$ (0.15 mol) and the aliphatic acids (**7–9**, 0.15 mol) was heated slowly with stirring till the solution became homogeneous. Appropriate phenol (**1–6**, 0.15 mol) was added and the reaction mixture was kept for about 2 h at 140–145°C. The reaction mixture was cooled, poured over crushed ice containing hydrochloric acid (1:1). The solid that separated out was filtered and washed separately with water and sodium bicarbonate solution. The crude product was purified using column chromatography over silica gel, elution was done with a gradient solvent system of petroleum ether/ethyl acetate to obtain the corresponding pure arylalkyl ketones **14–16** and **21–25** in 70–80% yields; these were characterized from their spectral data and comparison of the data with those reported in the literature [17–25].

Boron-trifluoride catalyzed condensation

A mixture of the acid **10–13** (1 mol) and resorcinol **1** (0.25 mol) was slowly saturated (2–3 h) with boron trifluoride at temperature ranging from 65–85°C. The reaction mixture was treated with water and the ketones **17–20** were obtained in good yields after recrystallization from appropriate solvent. All the ketones were characterized from their spectral data and the comparison of the data with those reported in the literature [17–25].

Synthesis of benzophenones

The synthesis of benzophenones was achieved under anhydrous conditions at room temperature by the Friedel-Crafts acylation of hydroxy/methoxy benzene derivatives **32–36** with the appropriately substituted benzoyl chloride **26–31** in the presence of aluminium chloride in ether. The hydroxy/methoxy benzene derivatives **32–36** (1 mmol), benzoyl chloride (0.5 mmol, 0.07 g) and anhydrous aluminium chloride (0.7 mmol, 0.094 g) were mixed and heated at 90°C for 4–5 h. The progress of the reaction was followed by TLC. The dark brown mixture was poured onto crushed ice containing conc. HCl (10 mL) and the resulting mixture was stirred at 70°C for 30 min. After cooling, the organic layer was separated, washed with water and dried over Na_2SO_4. The solvent was removed by distillation and the products **37–42** were recrystallized from a mixture of chloroform and petroleum ether. All the benzophenones were characterized from their spectral data and the comparison of the data with those reported in the literature [26–31].

Synthesis of desoxybenzoins

A mixture of the appropriate phenol **43–48** (1 mmol), phenyl acetic acid **49–54** (1 mmol) and $BF_3.Et_2O$ (5.1 mmol) was stirred at 85°C for 90 min. After completion of the reaction, the reaction mixture was poured into NaOAc solution (10%) and allowed to stand for 4 h. The product was filtered, washed with water and dried in air. The crude compounds were purified by column chromatography on silica gel using EtOAc/petroleum ether (1:10) to yield desoxybenzoins **55–64**. All these desoxybenzoins were fully characterized from their spectral data and the comparison of the data with those reported in the literature [32–40].

Syntheisis of different chalcones and their analogues/derivatives

*General procedure for the synthesis of 1,3-diarylpropenones (chalcones, **74–83**): Claisen-Schmidt condensation of acetophenones with aldehydes*

The appropriately substituted acetophenone **65–70** (5 mmol) was dissolved in ethanol (30 mL) and freshly fused $Ba(OH)_2$ (2 g) was added, followed by the addition of a solution of the corresponding aldehyde **71–73** (5 mmol) in ethanol (5 mL). The reaction mixture was refluxed for 6–7 h and progress of reaction was monitored on TLC (petroleum ether/ethyl acetate, 4:1). After completion of the reaction, the reaction mixture was poured onto crushed ice and the pH of the solution was made acidic using dilute hydrochloric acid. The solid obtained was filtered, dried and recrystallized from ethanol to give 1,3-diarylpropenones (chalcones) **74–83** in 75–86% yields. The compounds **74–83** were known in the literature and were fully characterized from their melting points, IR and NMR data and by comparison of the data with the data reported in the literature [41–51].

*Preparation of 1,3-diphenylpropanones **84–87***

A solution of 1,3-diphenylpropenone (chalcone) **80–83** (1 g, 3.5–4.5 mmol) in 15 mL of dry ethyl acetate was placed in the reaction bottle of atmospheric pressure hydrogenation apparatus and to that was added 0.1 g of palladium-charcoal (10%). The air was displaced with hydrogen and the mixture was shaken for 1 h. The progress of the reaction was monitored by TLC (petroleum ether/ethyl acetate, 4:1). After completion of the reaction, the palladium-charcoal was filtered and ethyl acetate was removed under vaccum. The solid obtained was purified by column chromatography to obtain the 1,3-diphenylpropanones **84–87** in 80–85% yields. The spectral data of all products were identical with those of compounds mentioned in the literature [52, 53].

*Preparation of 1,3-diphenylpropan-1-ols **88–91***

Powdered $NaBH_4$ (0.25 g) was added to a stirred solution of 1,3-diphenylpropanones **84–87** (1 g) in MeOH (50 mL) and the reaction mixture was stirred at room temperature for 5 min. After completion of reaction, MeOH was removed *in vacuo.* The oily residue was washed with $NaHCO_3$ and water, and the mixture was extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and concentrated to dryness to get compounds **88–91** as oils in 95–97% yields, the spectral data of all the products were found to be identical with those of the corresponding compounds mentioned in the literature [54–57].

*Synthesis of 1,3-diphenylpropenes **92–95***

To a solution of 1,3-diphenylpropan-1-ol **88–91** (1 g) in dioxane (30 mL), 5–7 drops of concentrated H_2SO_4 were added. The reaction mixture was refluxed for 4–5 h and the progress of reaction

was monitored by TLC in pure petroleum ether. After completion of reaction, the reaction mixture was poured over ice-cold water with vigorous stirring. The reaction mixture was extracted with petroleum ether and the combined organic layer was dried over Na_2SO_4 and concentrated to dryness to get the compounds **92–95** as oils in 85–90% yields, the spectral data of all the products were compared and found identical with those of the corresponding compounds mentioned in the literature [58–61].

*Synthesis of 1,3-diphenylpropanes **96–99***

A solution of 1,3-diphenylpropene **92–95** (1 g, 3.5–4.5 mmol) in 15 mL of dry ethyl acetate was placed in the reaction bottle of atmospheric pressure hydrogenation apparatus and to that was added 0.1 g of palladium-charcoal (10%). The air was displaced with hydrogen and the mixture was shaken for 1 h. The progress of the reaction was monitored by TLC (petroleum ether/ethyl acetate 4:1). After completion of the reaction, the palladium-charcoal was filtered and ethyl acetate was removed under vacuum. The solid obtained was purified by column chromatography to obtain 1,3-diphenylpropanones **96–99** in 85–90% yields. The spectral data of all the products were identical with those of the corresponding compounds reported in the literature [62–65].

Cells and cell culture

Primary endothelial cells were isolated from human umbilical cord using mild trypsinization [3]. The cells were grown in M-199 medium supplemented with 15% heat inactivated fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin B, endothelial cell growth factor (50 μg/mL). At confluence, the cells were subcultured using 0.0 5% trypsin-0.01 M EDTA solution and were used between passages three to four.

Cell viability assay

The cytotoxicity of different compounds was analyzed by using Trypan blue exclusion test and it was further confirmed by colorimetric MTT (methylthiazolydiphenyl-tetrazolium bromide) assay as described by us earlier [15]. Briefly, endothelial cells were treated with DMSO or these derivatives for 24 h. Four hours before the end of incubation, medium was removed and 100 μL of MTT (5 mg/mL in serum free medium) was added to each well. The MTT was removed after 4 h, cells were washed out with PBS, and 100 μL DMSO was added to each well to dissolve water insoluble MTT-formazan crystals. Absorbance was recorded at 570 nm in an ELISA reader (BIO RAD, Model 680, USA). All experiments were performed at least 3 times in triplicate wells. Maxium tolerable dose at which the cells were more than 95% viable was used for testing the compounds.

Modified cell-ELISA for measurement of ICAM-1

Cell-ELISA was used for measuring the expression of ICAM-1 on the surface of endothelial cells [15]. Endothelial cells were incubated with or without the test compounds at desired concentrations for the required period, followed by treatment with TNF-α (10 ng/mL) for 16 h for ICAM-1 expression. The cells were fixed with 1.0% glutaraldehyde. Non-specific binding of antibody was blocked by using skimmed milk (3.0% in PBS). Cells were incubated overnight at 4°C with anti-ICAM-1 mAbs, diluted in blocking buffer, the cells were further washed with PBS and incubated with peroxidase-conjugated goat anti-mouse secondary Abs. After washings cells were exposed to the peroxidase substrate (*o*-phenylenediamine dihydrochloride 40 mg/100 mL in citrate phosphate buffer, pH 4.5). Reaction was stopped by the addition of 2 N sulphuric acid and absorbance at 490 nm was measured using a microplate reader (Spectramax 190, Molecular Devices, USA). The percentage inhibition was calculated as $[\Delta C - \Delta D/\Delta C] \times 100$. Where, ΔC is delta control; optical density of TNF-α stimulated cells − unstimulated cells and ΔD is delta drug; optical density of drug treated and TNF-α stimulated cells − drug unstimulated cells.

Determination of IC_{50}

The percentage inhibitions of each compound at its various log concentrations were measured and plotted graphically from three independent experiemnts. From the graph plotted, the concentration at which a compound showed 50% inhibition was taken as its IC_{50} value.

Statistical analysis

Results are given as means ± SD. Independent two-tailed Student's *t* test was performed. Differences were considered statistically significant for $p < 0.05$. The statistical analysis was performed using Microcal Origin software (ver 3.0; Microcal Software Inc, Northampton, MA, and Cell Quest Software, Becton–Dickinson, USA).

Authors acknowledge the help of St. Stephen's Hospital, Delhi for providing the umbilical cord. This work was partly funded by the Council of Scientific and Industrial Research, India grant NWP0033 (to BG); the NIH grants HL081205, HL095420 (SCCOR), NIEHS-P50ES01590, GM079239, P50ES015903, P01 ES018176 and ES03819 (to SB); the University of Leuven (IDO06/006) & the F.W.O. (Fund for Scientific Research–Flanders, Belgium) grant (to EVDE); the University of Delhi grant under the Strengthening R&D Doctoral Research Programme and the Department of Scientific & Industrial Research (DSIR, New Delhi) to VSP, SKS and AKP.

The authors have declared no conflict of interest.

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