REVIEW
REVIEW OF LITERATURE
Review of Literature

Occurrence

Chalcones are the first isolable compounds from flavonoid biosynthesis in plants but do not necessarily accumulate to any appreciable degree unless the enzyme chalcone isomerase, which catalyzes the cyclisation of chalcone to flavanone is absent (Bohm, 1998). Most naturally occurring chalcones are polyhydroxylated (Bohm, 1975). Chalcones which are important flavour principles in citrus plants, occur widely in green plants (Harborne, 1967). Chalcones are found to be distributed throughout the plant kingdom.

According to Tomas-Barberan and Clifford (2000) chalcones have restricted occurrence in foods but both citrus fruits and apples are rich dietary sources of chalcones and dihydrochalcones and these compounds could even make a greater contribution to the total daily intake of natural polyphenolics than the more extensively studied flavonoids. The most common chalcones found in foods are phloretin
and its glucoside phloridzin (phloretin 2’-O-glucose), chalconaringenin and arbutin. Dihydrochalcone-phloretin and phloridzin are characteristic of apples. Chalconaringenin is characteristic of pears. Arbutin is also found in strawberry and bearberry, in wheat, in wheat products and in trace amounts in tea, coffee, red wine and broccoli (Robards et. al., 1999; Clifford, 2000). Chalconaringenin is present in tomato peel and may be present in tomato products (juice, paste and ketchup). The naringenin chalcone content of tomato skin in post-climacteric fruits is 64 mg⁻¹ kg⁻¹ FW but in tomato ketchup, naringenin chalcone is transformed to naringenin (flavanone), and the chalcone is present only in trace amounts (Macheix et. al., 1990). Muir et. al. (2001) observed that the chalconaringenin was absent in the green peel but increased sharply during coloring of the fruit. Neohesperidin dihydrochalcone is an intense sweetener and permitted for commercial use as a food additive in Europe (Gibney et. al., 1995). It may be used in a wide range of foods (non-alcoholic drinks, desserts and confectionary) at concentrations in the range of 10-400 mg kg⁻¹ (or mgL⁻¹) or as a flavour modifier at concentrations of upto 5mgkg⁻¹ (Lindley, 1996). A particularly rich source of prenylated chalcones are the inforescences of Humulus lupulus.
that are used in the brewing industry to flavor and bitterness to beer (Miranda, 2000).

Butein (2’4’,3,4-tetrahydroxylchalcone) is a major active component of Dalbergia odororera, have cytotoxic in addition to antioxidant and anti-inflammatory activities (Ramanathan, 1992; Yit & Das, 1994; Cheng 1998; Chan, 1998). It also prevents antiglomerular basement membrane antibody-associated glomerulonephritis in rats (Hayashi, 1996). Butein, a plant polyphenol, also is one of the major active components of the stems of Rhus verniciflua have been traditionally used for the treatment of pain, parasites, and thrombotic disease in Korea (Kang, 2003).

Isoliquiritigenin is a flavonoid with chalcone structure (2′,4′,4-trihydroxylchalcone), an active compound present in plants like Glycyrrhiza and Dalbergia which showed various biological activities including anti-inflammatory, anti-carcinogenic and antihistimic (Vaya, 1997). Also, 2’, 4’, 4- trihydroxychalcone (isoliquiritigenin) extracted from licorice root, is regarded as a promising lead potential cancer chemopreventive agent (Kinghorn, 2004).
Most floral colours present in nature arise from flavonoids (Brouillard and Dangles, 1993). In some species chalcones constitute the major yellow flower colour pigments. As chalcones are the initial intermediate used in the biosynthesis of all flavonoids, those with 2',4',6' trihydroxylation of the A- ring are the substrates for the production of anthocyanins and flavonols and those without a hydroxyl group at the 6' position are precursors of limited number of plant species, primarily in the leguminosae (Barz and Welle, 1992; Dixon and Pavia, 1995). Chalcones provide yellow pigmentation in the flowers of several ornamental species (Brouillard and Dangles, 1993). Yellow- flowered varieties of Dianthus caryophyllus (Forkmann and Dangelmayr, 1980), Callistephus chinensis (Kuhn et. al., 1978), and Cyclamen persicum (Miyajma, 1991). The yellow coloration of snapdragon flowers is provided by the aureusidin and bracteatin produced from 2',4',6',4- tetrahydroxychalcone or 2',4',6',3,4- pentahydroxychalcone (Sato, 2001).

Chalcones isolated from 'dragon blood', a resin obtained from the Dracaena cinnabari balf was evaluated for antioxidant activity (Macahala, 2001). Chalcones isolated from the leaf resin of Z. punctata, a shrub occurring in Western Argentina possess cytoprotective effects
(Rocha, 2003). Naringenin chalcone is the intermediate in flavonoid biosynthesis in Petunia (Heller and Forkmann, 1988, 1993). Interestingly, pollen of Mitchell Petunia is rendered yellow by a small amount of naringenin chalcone (Takamura and Miyajima, 1996). Butein-3-O- glucoside has only been reported in the yellow flowers of Butea monosperma (Gupta, 1970). Carthamin is the first natural chalcone provide red pigment to the flowers of Safflower (Carthamus tinctorium) and has the structure of 2’- glycosidoxy- 3’, 4, 4’, 6’- tetrahydroxychalcone (Sheshadri and Thakur, 1960). Yellow- flowered varieties of Cosmos, Dahlia, Coreopsis and Bidens (Bohm 1988, 1993) contains accumulated 6’- deoxychalcones.

**Chalcone Biosynthesis**

Chalcones are synthesized via the phenylpropanoid pathway. Phenylalanine ammonia lyase (PAL) catalyzes the conversion of phenylalanine to cinnamate. PAL also shows activity with converting tyrosine to p- coumarate, albeit to lower efficiency. The cinnamate 4-hydroxylase (C4H) catalyzes the synthesis of p- hydroxycinnamate from cinnamate and 4- coumarate: CoA ligase (4CL) converts to p- coumarate to coenzyme-A ester, activating it for reaction with the condensation of
one molecule of its 4- coumaroyl CoA, yielding naringenin chalcones (Stafford, 1991). This reaction is carried out by the enzyme chalcones synthase (CHS). Chalcone is isomerised to a flavanone by the enzyme chalcone flavanone isomerase (CHI). From these central intermediates, the pathway diverges into several side branches, each resulting in a different class of flavonoids.
Biosynthesis of Chalcones
**Metabolism and Excretion**

Although numerous metabolic studies have been reported on the fate of other flavonoids including flavonols, flavans, and flavanones in vivo but very few reports have been published on the metabolism of chalcone or chalcone derivatives in the mammal (Chanal et al., 1981) and the fate of chalcones in the mammalian systems is largely unknown.

Chalcones have been reported to undergo extensive metabolism by many routes, such as reversible conjugate addition of glutathione, demethylation of methoxy groups, oxidation of aromatic rings, and reduction of the enone moiety (Hawkins, 1980; Chabannes, 1973; Nozu, 1980; Brown and Griffiths, 1983). After oral or intravenous administration of 3,4- dihydroxychalcone like (2', 5'- dimethoxy-3, 4-dihydroxychalcone and 2'- ethoxy- 5'- methoxy- 3, 4- dihydroxy-chalcone), unchanged forms were undetectable in rat plasma using HPLC. These chalcones were rapidly and extensively metabolized after systemic administration (Sogawa, 1993). Butein (2',4',3,4- tetrahydroxychalcone), isoliquiritigenin (2',4',4 trihydroxychalcone), 2',3,4- trihydroxychalcone following administration to bile duct cannulated rats, 3'- O- methylbutein and 4'-O- methylbutein are selectively excreted in
the urine and bile as conjugates. Formation of demethylated products of 3-O- methylbutein and 4-O- methylbutein in vivo and vitro suggests that certain methylated chalcones excreted from the liver into the duodenum by the way of bile may be demethylated by the microflora in the gastrointestinal tract. Also administration of Butein; Isoliquiritigenin; 2’, 3, 4- trihydroxychalcone to the non- cannulated rats led to the presence of two sulphate conjugates of each chalcone in the faeces (Brown and Griffiths, 1983). Trace amounts of Butein in the bile of the rats dosed with isoliquiritigenin (2’, 4’, 4- trihydroxychalcone) indicate that liver metabolizes this chalcone by hydroxylation (Hackett, 1979),

<table>
<thead>
<tr>
<th>Compound</th>
<th>Period of collection (days)</th>
<th>Fraction (%) of administered dose</th>
<th>Total</th>
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<tr>
<td></td>
<td></td>
<td>Urine</td>
<td>Faeces</td>
</tr>
<tr>
<td>Butein (oral dose)</td>
<td>9</td>
<td>39.9 (32.2-48.1)</td>
<td>51.0 (37.7-68.5)</td>
</tr>
<tr>
<td>Butein (parenteral dose)</td>
<td>7</td>
<td>40.7 (33.4-49.0)</td>
<td>55.6 (46.4-61.0)</td>
</tr>
<tr>
<td>Isoliquiritigenin (oral dose)</td>
<td>7</td>
<td>56.3 (47.6-63.6)</td>
<td>40.0 (24.1-55.3)</td>
</tr>
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Excretion of radioactivity after administration of $^{14}$C- chalcones to non- cannulated rats

In a study by DuPont and co-workers (2002) with alcoholic apple cider, 21 % of the Phloretin (dihydrochalcone) was excreted in 24 h urine. According to Monge (1984), half of the intragastric dose of
phloretin was excreted in the urine, mainly within two days. In contrast several dihydrochalcones (not known to occur in foods) were excreted predominantly unchanged in the urine and faeces (Skjevrak, 1986). When rats were given phloridzin (phloretin 2’-O-glucose) at 5μM by cannula, inhibits the uptake of glucose by the active glucose transporter (SGLT1) in the small intestine and impairs the resorption of glucose in the kidney, causing glucosuria (Rodriguez, 1982). Dihydrochalcones were absorbed in the small intestine of rats following the conjugation and, thus, could be recovered intact in plasma (Crespy et. al., 2001 a,b; Glock et. al., 2001).

**Synthesis of Chalcones**

These compounds can be obtained by synthesis through methods as per Suzuki (Eddarir et. al., 2003) or aldolic condensation (or condensation of Claisen- Schimdt), with reagents, solvents and catalysts submitted to ultrasound (Li et. al., 2002) or using inorganic catalyst such as NaNO3/NP (natural phosphate) (Sebti et. al., 2001); or even by a simple and direct method of reacting acetophenones and aldehydes, with sodium hydroxide (NaOH) or potassium hydroxide (KOH) as catalyst and methanol or ethanol as solvents, at room temperature (Vogel, 1989).
Synthesis of chalcone by Claisen-Schmidt condensation of acetophenone and benzaldehyde

\[
\text{CH}_3\text{C}=\text{CH}-\text{CO}_2\text{H} + \text{H}_2\text{C}=\text{CH}-\text{CO}_2\text{H} \rightarrow \text{CH}_3\text{C}=\text{CH}-\text{CO}_2\text{H} + \text{H}_2\text{C}=\text{CH}-\text{CO}_2\text{H}
\]

Synthesis of chalcones by Suzuki coupling between activated benzoic acids and phenyl vinyl boronic acids

\[
\text{C}_6\text{H}_5\text{X} + \text{HO}\text{B} = \text{C}_6\text{H}_5\text{OH} \rightarrow \text{C}_6\text{H}_5\text{C}=\text{CH}-\text{CO}_2\text{H}
\]

\[
\text{benzoyl chloride} \quad (X = \text{Cl}) \quad \text{phenylvinyl boronic acid}
\]

Although these substances are associated with several different kinds of biological activities, they are also frequently employed for the synthesis of a great variety of important derivatives, including flavanones (Sagrera and Seone, 2005), Mannich bases (Pandeya et al., 1999), and aryloxypropanolamines (Satyanarayana et al., 2004), among others.
Also, reaction of chalcones with thioglycolic acid, 3-mercapto propanoic acid or thiosalicylic acid in hot toluene yield pharmacologically active carboxylic acid derivatives of dihydrochalcones (Levai, 1991).

![Chemical structure](image)

Like chalcones, dihydrochalcones have been reported to have a wide range of biological properties (Harborne and Baxter, 1999). Briot and co-workers reported the synthesis of dihydrochalcones which involved palladium-catalysed coupling of o-halophenols and 1-aryl-2-propen-1-ols (Briot et al., 2004).
Physicochemical Properties

Lopez et. al. (2001) and Rastelli et. al. (2000) analyzed the chalcones by rotating the bonds about the $\alpha$, $\beta$- unsaturated carbonyl (enone) linkage to identify the minimum energy conformation and confirmed the overall planarity and rigidity of the extended $\pi$ system. In compound 1 ring A had greater rotational flexibility and deviate from planarity to a greater extent than ring B. Thus delocalisation of electrons between ring A and the carbonyl group occurs to a lesser extent compared to electron delocalisation between ring B and the $\alpha$, $\beta$- unsaturated double bond.
<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
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<th>Torsion angles (°) determined by AMI method</th>
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<tr>
<td></td>
<td>R</td>
<td></td>
<td>Θ1</td>
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<tr>
<td>1</td>
<td>H</td>
<td>H</td>
<td>33.5</td>
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<tr>
<td>2</td>
<td>H</td>
<td>4-NO₂</td>
<td>0.43</td>
</tr>
<tr>
<td>3</td>
<td>H</td>
<td>2-NO₂</td>
<td>26.9</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>2,4-Cl₂</td>
<td>31.5</td>
</tr>
<tr>
<td>5</td>
<td>H</td>
<td>2,4-(CH₃O)₂</td>
<td>34.0</td>
</tr>
<tr>
<td>6</td>
<td>2,4-(Cl)₂</td>
<td>2,4-(Cl)₂</td>
<td>119.0</td>
</tr>
</tbody>
</table>

Ring substituents also affect the planarity of the rings A and B (Lopez et al., 2001). Bulky substituents like nitro or chloro at position 2 of ring B caused an expected sharp increase in Θ3 values, due to rotation of ring from planarity to minimize the steric effects of the ortho-substituent. The presence of a 2- methoxy substituent caused only a small change in Θ3, indicating that the steric hindrance caused by the methoxy group was less compared to nitro or chloro. When ortho- chloro substituents were introduced on both rings A and B, there was a large increase in Θ1 compared to Θ3, and thus ring A was more susceptible to distortion than ring B.

When a 2’- hydroxyl group was introduced on ring A, intramolecular hydrogen bonding between the hydroxyl and the carbonyl
oxygen gave rise to a stable planar conformation (Rastelli et al., 2000). The Cα- Cβ double bond in the enone moiety of chalcones can adopt Z or E configuration. Almost all chalcones isolated in E- form and can be isomerised to the Z form by exposing methanolic solution of the chalcone to normal light (Iwata et al., 1997). The Z isomer showed more potent antitumourigenic activity than E form. Photoisomerisation of the E isomer to the Z isomer may cause changes in biological activity, thus it is prudent to protect solutions of chalcones from light.

Besides E/Z isomerism, carbonyl and Cα- Cβ double bond in chalcones were positioned cis with respect to each other (Ducki et al., 1998). The s- cis conformer was more stable than s- trans conformer. Introduction of a methyl group to the Cα position, altered the carbonyl and Cα- Cβ double bonds to a trans orientation. The α- methyl group also caused significant loss of planarity between ring A and the enone (θ1 56- 88°). It contribute to the enhanced biological activity of α- methylchalcones which were found to have greater cytotoxic effect against a human leukemia cell line than the unsubstituted analogues.
s-cis conformation  s-trans conformation

Also, the $\alpha$, $\beta$-unsaturated carbonyl entity in chalcones is a soft electrophile, and attract soft nucleophiles like thiols, rather than hard nucleophiles like amino and hydroxyl groups (Dimmock et. al., 1999). On the other hand, chalcones, which are typical Michael reaction acceptors, will react readily with glutathione (GSH), the most abundant intracellular non protein thiol.

Sabzevari and co-workers (2004) reported that some hydroxy-chalcones depleted the GSH content of rat hepatocytes accompanied by
a concurrent increase in oxidized glutathione (GSSG) for some chalcones. Oxidation of GSH to GSSG may be mediated by phenoxy radicals produced from hydroxychalcones. Alternatively, the chalcone may be metabolized to catechols and quinones, which react readily with GSH to form conjugates. Christenson (1999) also concurred on the role of GSH depletion in chalcone- induced toxicity.

According to Lopez et. al. (2001), presence of electron withdrawing groups on ring B enhance the electron deficiency of the β-carbon, and thus its reactivity to nucleophiles. Similarly, the electron deficient carbonyl carbon is made more electron poor when electron withdrawing groups are present on ring A. Electron donating groups on either rings A and B will have the opposite effect.

Alston and Fry (2004) investigated that the reduction of the carbonyl group was facilitated by the presence of electron withdrawing groups and influenced to a greater extent by the inductive effect of the substituent.

The hydroxyl substituent is widespread among chalcones from natural sources and synthesized chalcones. Rastelli et. al. (2000)
concluded that 4’- hydroxyl was the most acidic (pKa 7.5) while 2’- hydroxyl was the least acidic.

![Chemical structure](10.1) OH O
(7.5) HO
(8.5) OH

**Effects on Mammalian Enzyme Systems**

**Lipoxygenase and cyclooxygenase activity**

Arachidonic acid released from membrane phospholipids or other sources is metabolized by the lipoxygenase and cyclooxygenase pathway to the smooth muscle contractile and vasoactive leukotrienes, LTC₄, LTD₄ and LTE₄ as well as LTC₄ (Lewis et. al., 1990). These molecules are intimately involved in inflammation, asthma and allergy, as well as other physiologic and pathologic processes.

Sogawa et. al. (1993) suggested that 3, 4- dihydroxychalcones (2’,5’-dimethoxy-3,4- dihydroxy- and 2’- ethoxy-5’- methoxy-3,4-dihydroxy) possessed potent anti-5-lipoxygenase and anticyclo-oxygenase activities by means of their antioxidative activities and are
stronger inhibitors than caffeic acid, nordihydroguaiaretic acid and quercetin. They also inhibited arachidonic acid-induced mouse ear edema. 2′,5′-dihydroxychalcone and 3,4- dihydroxychalcone had potent anti-cyclooxygenase and anti-inflammatory activity (Lin et al., 1997).

According to Nakadate and coworkers (1985) hydroxychalcone derivatives are inhibitors on mouse epidermal 12-LO and CO. In addition 3′,4′,5′,3,4,5-hexamethoxychalcone inhibits the prostaglandin E₂ (PGE₂) and nitric oxide by inhibiting the induction of COX-2 and inducible nitric oxide synthase in LPS-stimulated mouse peritoneal macrophages (Herencia et al., 1999). 2′- hydroxychalcone derivatives (2′,4′- dihydroxy- 4′-methoxychalcone ; 2′,4′- dihydroxy- 6′-methoxychalcone ; 2′- hydroxy- 4′-methoxychalcone) were potent inhibitors of TPA-induced prostaglandin E₂ production and inhibited COX-1 (Kim et al., 2001).

**Estrogenic activity**

A number of polyhydroxylated chalcones possess estrogenic properties (Miksicek, 1995). The chalcone skeleton is structurally similar to adrenal androgens and estrogens and their bisphenolic structure makes them
particularly interesting for interaction with receptors and enzymes such as aromatase 3β-HSD and 17β- HSD.

Bail et. al. (2001) reported for the first time that chalcones are potent inhibitors of aromatase and 17β- hydroxysteroid dehydrogenase, enzymes involved in the biosynthesis of estrogens, but found to be weaker inhibitors compared to flavones and flavanones. The most potent inhibitor of aromatase was naringenin chalcone and eriodictyol chalcones (IC₅₀ 2.6 and 2.8 μM respectively) (Blanco et. al., 1997), while 4- hydroxychalcone (IC₅₀ 16 μM) was the most potent inhibitor of 17β- HSD. Di- and tri-hydroxylated chalcone derivatives (4,4'-dihydroxychalcone; 2,4,4'- trihydroxychalcone; Phloretin) are able to bind to the human estrogen receptors at micromolar concentrations and to serve as effective estrogen agonists (Miksicek, 1993). Non-hydroxylated chalcones exhibited weak binding to estrogen receptor (ER) (Branham et. al., 2002). Multi- hydroxylated chalcones (2',4',4'- trihydr- roxychalcone ; 4,2',4',6'- tetrahydroxychalcone) competed with [3H]- E₂ for ER but with lower affinity than E₂ (17β- estradiol). Also 3'-methyl-3- hydroxychalcone inhibit the binding of estradiol to type- II estrogen binding sites in HGC-27 cells (Satomi, 1993).
Dihydrochalcones and methoxylated chalcones had significantly reduced inhibitory activity (Lerner et. al., 1963).

Although these estrogentic flavonoids are less potent than 17β-estradiol, they appear to possess a pharmacological efficacy equivalent to that of the biological hormone.

**Inhibition of aldose reductase**

Aldose reductase is a key enzyme of polyol pathway, catalyzes NADPH-dependent reduction of glucose to sorbitol and an excessive accumulation of intracellular sorbitol is considered responsible for cataractogenesis, retinopathy, neuropathy of diabetic origin (Kador et. al., 1985).

Severi (1996) tested a series of hydroxy and hydroxy-methoxychalcones towards bovine lens aldose reductase (AR) and showed that 4, 4′- dihydroxychalcon; 2′,4′- trihydroxychalcone; 4-hydroxy- 4′- methoxychalcone and 4- hydroxy- 2′,4′-dimethoxychalcone displayed a good level of inhibitory activity. A series of 2′, 4′, 4′-trihydroxychalcone containing modifications on aromatic rings display affinity for aldose reductase enzyme (ALR₂) (Aida et. al., 1990). Lim et. al. (2001) tested flavonoid derivatives on rat lens AR and their
antioxidant effects using Cu\(^{2+}\) chelation and radical scavenging activities in vitro, 2,4, 2', 4'-tetrahydroxy-; 2, 2', 4'-trihydroxy-; 2', 4'-dihydroxy-2,4-dimethoxy-; 3,4, 2', 4'-tetrahydroxychalcone possess good inhibitory activity. Also, butein was the most potent promising compound for the treatment of diabetic complications. Hesperidin chalcone shows 82% inhibition of aldose reductase and polyol accumulation in rat lenses incubated in sugar xylose at a motor concentration of 10\(^{-4}\) (Varma and Kinoshita, 1976).

**Tyrosinase inhibition**

Tyrosinase inhibitors could prove to be effective depigmenting agents. The tyrosinase enzyme is involved in two reactions in the melanin biosynthesis pathway (Briganti et. al., 2003). Isoliquiritigenin (2', 4', 4'-trihydroxychalcone) can inhibit both mono- and di-phenolase tyrosinase activities at IC\(_{50}\) 8.1 mM and the effect of isoliquiritigenin was dose dependent (Nerya et. al., 2003). Khatib et. al. (2005) showed that 2, 4, 3', 4'-tetrahydroxychalcone and 2, 4, 2', 4'-tetrahydroxy chalcone are very active tyrosinase inhibitors.
Mitochondrial enzymes

A number of chalcones inhibit various enzymes located in the mitochondria. 2’-Hydroxychalcone and derivatives (principally methoxy and hydroxy) uncoupled oxidative phosphorylation in mitochondria isolated from mung bean and potato mitochondria (Ravanel, 1982). Included among this group of uncouplers were the naturally occurring chalcones, isoliquiritigenin and butein. The dihydrochalcone phloretin also demonstrated uncoupling activity.

Low concentrations of a number of chalcones stimulated respiration in rat liver mitochondria which was due to their uncoupling activity (Takashi et al., 1984). However at higher concentrations the electron transport chain was inhibited.

Butein and various related polyhydroxylated flavonols and other flavonoids inhibited succinoxidase activity in beef heart mitochondria (Hodnick et al., 1986). Butein and luteolin, which is a flavone having a similar configuration of hydroxy groups as Butein had the greatest potencies, suggesting the importance of the number and position of hydroxy groups.
Chalcones interfered with respiration and oxidative phosphorylation as well as membrane permeability of isolated rat liver mitochondria (Inoue et. al., 1982). Isoliquiritigenin isolated from the roots of Glycyrrhiza uralensis inhibited mitochondrial monoamine oxidase from rat liver (Tanaka et. al., 1987). It displayed a substrate-competitive inhibition of monoamine oxidase having a $K_i$ value of 2.11 μM.

**Anti Inflammatory Property**

Mast cells play a central role in the pathogenesis of diseases such as allergic asthma, rhinoconjunctivitis, urticaria, anaphylaxis and in chronic inflammatory disorders (Middleton & Kandaswami, 1992). The neutrophil is an important inflammatory cell and can be triggered by a variety of inflammatory stimuli to produce highly reactive oxygen species which have potent microbicidal and inflammatory effects (Babior, 1978; Tauber and Babior, 1985).

Study by Hsieh (1998) showed that chalcones (2', 3- dihydroxychalcone; 2'-hydroxy- 3, 4- dimethoxychalcone ; 3'- hydroxy-3,4-dimethoxychalcone ; 2',5'- dihydroxy-3,4,- dimethoxychalcone) caused strong and dose dependent inhibition of mast cell degranulation and also
indicated potent inhibitory effects on the release of β-glucoronidase and lysozyme from neutrophil degranulation caused by fMLP/CB. Also 2’, 5’-dihydroxy-3, 4- dimethoxychalcone showed enhancement of inhibitory effects on histamine release from mast cell degranulation. In another study, Hsieh et. al. (2000), showed that chalcones caused mast cells degranulation inhibition and inhibitory effects on the activation of neutrophils and also that enone moiety of chalcones appears to be required for the inhibition of mast cell degranulation and superoxide anion formation from rat neutrophils. Lin et. al. (1997) showed that chalcones caused inhibition of mast cells and neutrophils and release of β-glucoronidase and lysozyme from neutrophil degranulation. Dihydrochalcone Phloretin inhibited the histamine release from human basophils (Grossman, 1988).

Interleukin-1 is liberated when the body reacts to such injurious stimuli as microorganisms and antigens and it causes and exacerbates inflammatory conditions. 2’, 4’, 6’-trimethoxychalcone exerted a potent inhibitory action towards the biosynthesis of interleukin-1 (Batt, 1993).

2’-hydroxychalcone downregulates the TNF-α and LPS induced expression of ICAM-1 and VCAM-1 in human umbelical vein
endothelial cells via inhibition of the alteration of NF-kB (Madan et. al., 2000). Ban et. al. (2004) showed that 2′- hydroxychalcone derivatives (2′-hydroxy-4-dimethoxychalcone; 2′,4-dihydroxy-4′dimethoxychalcone; 2′,4-dihydroxy-6-dimethoxychalcone) suppressed the LPS production of nitrite and TNF-α in the macrophage cell line during the inflammation process. Isoliquiritigenin has been reported to suppress cyclooxygenase-2, known to play an important role in inflammation (Takahashi et. al., 2000) and also blocked pro-inflammatory cytokine-induced expression of VCAM-1, E-selectin through NF-KB signal disruption (Kwon et. al., 2007). Butein suppressed the nuclear factor (NF) kB activation induced by various inflammatory agents and also the expression of cyclooxygenase (Pandey et. al., 2007).

Platelets are also inflammatory cellular elements (Weksler, 1983; Metzger and Page, 1998). Chalcones are potent inhibitors of arachidonic acid – induced platelet aggregation in rabbit platelets and secondary aggregation induced by epinephrine in human PRP.

**Cytotoxic Effects**

A variety of chalcones possess cytotoxicity towards a number of cell lines (Wattenberg, 1994; Edwards, 1988; Yit & Das, 1994; Cheng,
Unsubstituted chalcone displayed minimal activity towards human colon cancer adenocarcinoma cell line 220.1 having an IC$_{50}$ greater than 100$\mu$M. Butein significantly inhibited the incorporation of $[^{14}\text{C}]$-thymidine, uridine and leucine into colon 220.1 cells at a concentration of 2$\mu$M by inhibiting the DNA, RNA and Protein synthesis (Yit & Das, 1994) while drug 5-fluorouracil used in the chemotherapy of colon cancer (Moertal, 1980) inhibited incorporation of $[^{14}\text{C}]$-uridine at a concentration of 50$\mu$M. Butein also displayed potent cytotoxicity towards Raji lymphoma and Hela cell lines (Ramanathan, 1993). Several polyhydroxylated chalcones, particularly isoliquiritigenin have shown TPA-induced tumor promotion of the mouse epidermis (Yamamoto et. al., 1991). 3'-methyl-3-hydroxychalcone has been reported to be a potent inhibitor of proliferation of several cell lines of malignant human cells (pancreatic, gastric, neuroblastoma, cervical) in vitro and to suppress TPA-induced tumor promoting activity in mouse epidermis in vivo. This chalcone also inhibit BaP-induced pulmonary edenoma formation in female A/J mice (Satomi, 1993). 4'-methoxychalcone was found to inhibit the formation of pulmonary tumors (Wattenberg, 1995). Makita et. al. (1996) demonstrated that dietary adminstration of chalcone, 2'-hydroxychalcone during post-
initiation phase effectively suppresses 4-NQO-induced oral carcinogenesis without any toxicity. 2-hydroxychalcone can effect proliferation of Hela cells by initially inhibiting DNA and RNA (Ramanathan et. al., 1993). Chalcones with carboxylic acid substituents were shown to bind to the tryptophan pocket of the p53 binding site of MDM2 (mouse double minute 2) oncogene and to promote dissociation of the p53/MDM2 complex (Stoll, 2001). The MDM2 oncogene is over-expressed in human breast cancer. Thus, disruption of p53/MDM2 complex is considered an attractive target in cancer therapy.

The naturally occurring chalcone calythropsin and its dihydro analogue demonstrated differential cytotoxicity towards a variety of human tumour cell lines (Beutler et. al., 1993). Naturally occurring hydroxychalcones prevented gastric mucosal lesion formation induced by necrotising agent suggesting cytoprotective property on gastric mucosa (Yamamoto et. al., 1992). 2′, 4′-dihydroxychalcone and 2′,4′-dihydroxy-3′-methoxychalcone showed significant cytoprotective effects in gastroduodenal tract in rats (Rocha et. al., 2003). A series of 2′, 5′-dihydroxy chalcones were reported for cytotoxic activity against a
variety of tumor cell lines as well as non-tumor endothelial cell line (Nam et al., 2003).

One of the most widely cited mechanism by which chalcones exert their cytotoxic activity is that of interference with the mitotic phase of the cell cycle. Edwards and coworkers (1990) found a large number of methoxylated chalcones with anti-mitotic activity against HeLa cells. Ducki et al. (1998) investigated α-substituted chalcones as the most active chalcones against K562 human leukemia cell line. 2'-oxygenated chalcone derivatives showed potent inhibitory activity toward Jurkat cell line (Rao, 2004).

Angiogenesis, the generation of new capillaries from pre-existing vessels is enhanced in pathological conditions, 2'-hydroxy-4'-methoxychalcone (HMC) decreased angiogenesis in both chick embryos in the chorioallantoic membrane assay and basic fibroblast growth factor-induced vessel formation in the mouse matrigel plug assay. 4',4'-dihydroxychalcone, investigated for inhibition of human myeloid HL 60 leukemia cells, caused changes in the ladder assay, suggestive of apoptosis (Saydam et al., 2003). Compound, 2-chloro-2', 5'
dihydroxychalcone also had good activity against the various cell lines (Lopez et. al., 2000).

A recent study proposed that the cytotoxicity of hydroxychalcones may be due to in part to their ability to uncouple mitochondrial respiration, thus causing collapse in mitochondrial membrane potential (Sabzevari et. al., 2004). The authors noted that chalcones with fewer hydroxyl groups on ring A and B were more effective as compared to chalcones with more hydroxyl groups.

**Chemoprotective Chalcones**

Chemoprotection by chalcones may be a consequence of their antioxidant properties mediated via induction or inhibition of metabolic enzymes, by exerting an anti-invasive effect or a reduction in nitric oxide production.

**I) Antioxidant chalcones**

Free radicals are involved in different stages of carcinogenesis. Chalcones with good antioxidant activities were cytotoxic against tumor cell lines and reduced ascites tumour development in mice (Anto et.al, 1995). Naringenin chalcone and phloretin showed to active scavenger of DPPH and hydroxyl free radicals, but no anti-proliferative activity was
observed against cancer cell line (MCF-7), while 2'-hydroxychalcone showed a biphasic response—anti-proliferative activity at high concentration and promoting cell growth at lower concentration (Calliste et. al., 2001). Several 2'-hydroxy substituted chalcones possess low antioxidant activity but are good inhibitors of CYP1A (Machala, 2001). Dihydrochalcone phloretin is a potent antioxidant in peroxynitrite scavenging and the inhibition of lipid peroxidation (Rezk et. al., 2002).

II) Inhibition of induction of metabolizing enzymes

Cytochrome P450 enzymes activate a number of procarcinogens to reactive intermediates that subsequently interact with cellular nucleophiles to trigger carcinogens. Hydroxy and methoxy substituted chalcones were found to be good inhibitors of CYP1A activities in hepatic microsomes isolated from mice (Machala, 2001). In another study by Monostory (2003), methoxylated chalcones (4-methoxychalcone; 3-methoxy-chalcone; 2-methoxychalcone) were found to be good inhibitors of CYP1A- dependent metabolism of ethoxyresorufin.

The induction of phase 2 enzymes like GST and NAD(P)H:quinone reductase (NQO1) is an effective chemoprotective mechanism.
The moderate inducer potency of the unsubstituted chalcone (1,3-diphenylprop-2-en-1-one, CD 31 μM) was markedly increased upon ortho hydroxylation of either rings A and B (2'-hydroxychalcone, CD 9.8 μM; 2-hydroxychalcone, CD 12 μM) and enhanced even further when both rings carried ortho hydroxyl groups (2, 2'-dihydroxychalcone, CD 4.7 μM) (Dinkova-Kostova, 1998) suggesting that the ortho hydroxyl group may increase the reactivity of the reacting sulfhydryl group, possibly by mechanism(s) involving the inductive and hydrogen bonding effects of the hydroxy group (Dinkova-Kostova, 2001).

**III) Anti-invasive chalcones**

Prenylated chalcones have anti-invasive properties based on an in vitro test in which fragments of normal embryonic chick heart were confronted with invasive human mammary carcinoma cells (Mukherjee, 2001). Anti-invasive properties may involve interference with the formation of cellular protein complexes, involved in invasion (Parmar, 2003).
IV) Inhibition of nitric oxide- The free radical nitric oxide (NO) is an important effector of macrophage induced cytotoxicity (Laskin, 1995), and stimulates the production of inflammatory mediators such as TNF-α and interleukin-1β. Some chalcones inhibited NO production in lipopolysaccharide (LPS) and interferon-activated microglial cells. These chalcones with methoxy substituents on ring B and fluorinated substituents on ring A, inhibited the expression of inducible nitric oxide synthase (iNOS), the enzyme responsible for NO production. 2’, 5’-dihydroxychalcones inhibited NO production in LPS-induced macrophage, through the expression of iNOS protein expression (Ko, 2003) and thus showed anti-inflammatory effects.

Madan (2000) reported that 2’-hydroxychalcone downregulates TNF-α and LPS-induced expression of ICAM-1 and VCAM-1 in human umbilical vein endothelial cells via inhibition of the activation of NF-KB. The synthetic chalcone 3’,4’,5’,3,4,5-hexamethoxychalcone is an inflammatory compound able to reduce nitric oxide (NO) production by inhibition of iNOS protein synthesis (Alcaraz, 2004).
Miscellaneous Properties

Antibacterial properties

Chalcone and analogues in which hydroxy and methoxy groups were placed in the aryl rings were evaluated for antibacterial activity using Staphylococcus aureus (Pappano et. al., 1985). Chalcone and various dihydrochalcones inhibited bacterial growth and in particular 2′,4′-dihydroxy- and 2′,4- dihydroxy chalcone were antibacterial at low concentrations. Introduction of an additional hydroxy group in 2′-hydroxychalcone increased antibacterial activity, however the addition of methoxy groups to 2- hydroxychalcone led to a reduction in bioactivity (Pappano et. al., 1994; Devia et. al., 1998).

The introduction of alkyloxycarbonylmethoxy groups in the 3 or 4 position of ring B, led to a series of compounds with activity against both Gram- positive and Gram- negative bacteria (Szajda, 1989).

Antifungal properties

Chalcone have demonstrated toxicity not only towards bacteria but to fungi as well. Thus the introduction of methoxy or 2-diethylaminoethoxy groups into ring A of chalcones produced a group of compounds having bactericidal and fungicidal activity (Sangwan et. al.,
1983). In another study hydroxy, methoxy or benzyloxy substituents displayed activity towards phytopathogenic bacteria and fungi.

A number of chalcones possess antifungal activity particularly when one or more hydroxy groups are present in the aryl rings (Sato, 1995). 2-hydroxychalcones such as 2',4',4'-trihydroxychalcone; 5'-methyl-2',4,4'-trihydroxychalcone; 2,5'-dihydroxy chalcone inhibited the growth of eleven microorganisms of the Candida genus at a concentration of 100 μg/ml.

A report by Bilgin and co-workers (1987) revealed that 4'-hydroxychalcone was the active compound with comparable activity to oxiconazole against some of the fungi. Among a series of chalcones evaluated for antifungal activity 2'-OH; 4-CH3 chalcone; 2'-OH, 5'-CH3 chalcone; 3'-OH, 5-CH3 chalcone displayed the highest potency.

**Antiviral properties**

The chalcone (2'-hydroxy, 4,6'-methoxy, 4'-ethoxychalcone) is a potent inhibitor of rhinovirus uncoating. This chalcone acts synergistically with other antiviral agents such as dichloroflavan and enviroxime against the
human rhinovirus type 9 (Ahmad and Tyrell, 1986) but it was ineffective against human rhinovirus 2 (Al-Nakib et. al., 1987).

Licochalcone A inhibited giant cell formation in OKM-1 cells which were infected with the human immunodeficiency virus (Hatano, 1988). Hydroxy and methoxy chalcones were effective against the tomato ringspot virus on the leaves of Chenopodium quinoa (Onyilagha et. al., 1997)

**Antiprotozoal and insecticidal properties**

Leishmaniasis is a group of disease caused by a number of protozoa of the genus Leishmania. Licochalcone A demonstrated high activity towards the promastigote and amastigote stages of both Leishmania major and Leishmania donovani (Chen et. al., 1993). Licochalcone A destroyed the ultrastructure of Leishmania parasite mitochondria and inhibited the respiration and the activity of mitochondrial dehydrogenases of Leishmania parasite (Zhai et. al., 1995).

The two chalcones exhibited inhibitory effects on the activity of FRD in L. major promastigotes. FRD, one of the enzyme of respiratory chain, might be the specific target for the chalcones tested (Chen et. al., 2001). 2’,6’- dihydroxychalcone-4’-methoxychalcone showed significant
activity in vitro against promastigotes and intracellular amastigotes of L. amazonensis (Torres- Santos et. al., 1999). Substitution containing chalcones exhibited promising leishmanicidal and trypanocidal activities with no cytotoxic effect on mouse macrophages (Lunardi et. al., 2003).

A series of chalcones displayed toxicity towards the larvae of the mosquito Culex quinquefasciatus (Das et. al., 1995). A number of chalcones and related were evaluated for molluscicidal activity using the snail Biomphalaria glabrata (Adewunmi et. al., 1987). The chalcones displayed high potency while the epoxides were either inactive or possessed very weak molluscicidal properties.
GENERAL PROCEDURES FOR
PREPARATION OF CHALCONES USED IN THE STUDY
General Procedures for Preparation of Chalcones and their Dihydro-thio Derivatives

General Procedures

Reagents and solvents were of commercial grade and were used without further purification. All melting points were determined on a Kofler ho plate apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 621 spectrophotometer. $^1$H-NMR spectra were recorded in CD$_3$ OD or DMSO-d$_6$ solution with Bruker AC 300 operating at 400 MHz with tetramethylsilane used as internal standards. $^{13}$C-NMR was recorded on a varian Unity 75 and 100 spectrophotometers. Chalcones used in this study were prepared by following methods:

Preparation of 2', 4', 4-Trihydroxychalcone (ISL, isoliquiritigenin):

2', 4', 4-Trihydroxychalcone was prepared by the method of Severi et. al. (1996). 2-Hydroxy-4 (tetrahydropyran-2-yloxy) acetophenone (1gm, 4.24 mmol), 4-(tetrahydropyran-2-yloxy) benzaldehyde (2 eq.) and barium hydroxide octahydrate (2 gm, 6.3 mmol) were dissolved in
methanol (50mL) and stirred for 12h at 50°C. The reaction mixture was concentrated in vacuo. After water (100mL) was added to mixture, it was neutralized with 1M HCl and extracted with ethyl acetate. The organic layer was separated, washed, dried and evaporated in vacuo. The residue yielded crude 2'-hydroxy-4'-(tetrahydropyran-2-yloxy) chalcone. The compound was suspended in methanol (30 mL) and P-tolune sulfonic acid (0.02 g, 0.1 mmol) was added. The reaction mixture was stirred for 3 h at room temperature and then evaporated in vacuo. After water (30mL) had been added to the mixture it was neutralized with sodium carbonate and extracted with ethyl acetate. The organic layer was separated, washed with water, dried and evaporated in vacuo. The residue was chromato- graphed by MPLC (chloroform/ methanol/ formic acid 5: 1: 0.1) to give 2', 4', 4-trihydroxychalcone.

Physical form: yellow-orange crystalline solid. Yield: 88%, m.p: 210-211°C.

The structure of ISL has been established by 1H-NMR and 13C NMR.

1H-NMR (200.13MHz, CD3OD)δ 6.26 (d, J 2.3 Hz, H3'); 6.39 (dd, J 8.9, 2.3 Hz. H5') 7.96 (d, J 8.9 Hz, H6'); 7.6 (d, J 154 Hz, Ha); 7.78 (d, J 15.6
Hz, H₆); 7.61 (d, J 8.6 Hz, H₂); 6.83 (d, J 8.6 Hz, H₃); 6.83 (d, J 8.6 Hz, H₅); 7.61 (d, J 8.6 Hz, H₆)

¹³C NMR (50.33 MHz, CD₃OD): δ 103.8 (C₃'), 109.2 (C₅'), 114.6 (C₁), 116.5 (C₅), 116.9 (C₃), 118.3 (C α), 122.8 (C₁), 131.8 (C₂), 131.1 (C₆'), 145.6 (C β), 166.4 (C₄'), 165.7 (C₂'), 161.5 (C₄), 193.5 (C=O, β')

2', 4', 4-Trihydroxychalcone

**Preparation of 2', 4', 3, 4- Tetrahydroxychalcone (BUT, butein)**

2', 4', 3, 4- Tetrahydroxychalcone was prepared by the method of Sogawa et. al. (1993). 2,4 Dihydroxyacetophenone (3.8 g, 25 mmol) and pyridinium p- toluenesulph- onate (0.15 g, 0.6 mmol) were stirred for 0.5 h in dichloromethane (80 mL) and then 3,4-dihydro-α-pyran at room temperature for 4h. The reaction mixture was washed twice with water, dried, and evaporated in vacuo. The residue yielded crude 2, 4-bis (tetrahydropyran-2-yloxy) acetophenone (1a) or 3, 4-bis (tetrahydro-
pyran-2-yloxy) benzaldehyde (1b), respectively. Crude 1a, crude 1b, and barium hydroxide octahydrate (8.15 g, 25 mmol) were dissolved in MeOH (100 mL) and the mixture was stirred for 12 h at 40°C and then evaporated in vacuo. Water (100 mL) was added and the mixture was neutralized with HCl (1M, 35 mL) and extracted with EtOAc. The organic layer was separated, washed with water, dried, and evaporated in vacuo. The residue yielded crude 2', 4', 3, 4-bis (tetrahydro- pyran-2-yloxy)chalcone (1c). Crude 1c and p-toluenesulphonic acid (0.2 g, 1.05 mmol) were dissolved in MeOH. The reaction mixture and stirred for 4 h at room temperature and then evaporated in vacuo. Water (100 mL) was added and the mixture was neutralized with 5% NaHCO₃ (50 mL) and extracted with EtOAc. The organic layer was separated, washed with water, dried, and evaporated in vacuo. The residue was chromatographed on silica gel with cyclohexane-EtOAc (2:1) as mobile phase to yield 2', 4', 3, 4- tetrahydroxychalcone.

Physical form: Yellow solid. Yield: 72%, m.p: 251-252°C

The structure of this compound has been established by IR, ¹H-NMR and ¹³C-NMR spectra.

IR (KBr)v: 3300, 1650 cm⁻¹
\[ ^1\text{H-NMR (200 MHz, CH}_3\text{OD)} \delta 7.10 \text{ (d, 1H, J 2.4 Hz, H-3'), 6.42 (dd, 1H, J 2.4, 9.0 Hz, H-5'), 6.82 (d, 1H, J 8.2 Hz, H-5), 7.12 (dd, 1H, J 2.0, 8.2 Hz, H-6), 7.19 (d, 1H, J 2.0 Hz, H-2), 7.53 (d, 1H, J 15.3 Hz, H-\alpha), 7.73 (d, 1H, J 15.3 Hz, H-\beta), 7.94 (d, 1H, J 9.0 Hz, H-6') \]

\[ ^{13}\text{C-NMR (100 MHz, CD}_3\text{OD)} \delta 104.1 \text{ (C-3'), 109.5 \text{ (C-5'), 115.0 \text{ (C-1')}, 116.1 \text{ (C-2), 116.9 \text{ (C-5), 118.6 \text{ (C-\alpha), 123.9 \text{ (C-6), 128.7 \text{ (C-1), 133.3 \text{ (C-6')}, 146.3 \text{ (C-\beta), 147.1 \text{ (C-3), 150.2 \text{ (C-4), 166.6 \text{ (C-2'), 167.7 \text{ (C-4'), 193.8 \text{ (CO)}} \right) \]

\[
\begin{align*}
\text{HO} & \quad \text{HO} \quad \text{OH} \\
\text{OH} & \quad \text{K} \quad \text{OH}
\end{align*}
\]

**Preparation of 2-, 2- Dihydroxychalcone (DHC)**

2-, 2- Dihydroxychalcone was prepared by the method of Hsieh et. al. (1998). 2-hydroxyacetophenone (3.4g, 25 mmol), 2-hydroxybenzaldehyde (3.05 g, 25 mmol) and barium hydroxide octahydrate (8.15 g, 25 mmol) were dissolved in MeOH (100mL). The reaction mixture was
stirred for 12 h at 40°C and then evaporated in vacuo. Water (100mL) was added and the mixture was neutralized with HCl (1M, 35 mL) and extracted with ethylacetate. The organic layer was separated, washed with water, dried and evaporated in vacuo. The residue was eluated through a silica gel column with cyclohexane-ethylacetate (4:1) to give 2-, 2- dihydroxychalcone.

Physical form: Yellow amorphous solid, Yield: 38%, m.p: 160-162°C.

The structure of DHC was determined by IR, $^1$H NMR, $^{13}$C NMR

IR (KBr)v: 3256, 1636 cm$^{-1}$

$^1$H-NMR (200 MHz, DMSO-d$_6$) δ 12.62 (1H s OH-2'), 8.17 (1H, d, J 8Hz, H-6), 8.16 (1H, d, J 15.5 Hz, H-β), 7.97 (1H, d, J 16Hz, H-α), 7.90 (1H, d, J 8Hz, H-6'), 7.55 (1H, dt, J 1, 8.5Hz, H-4), 7.30 (1H, dt, J 1, 8Hz, H-5), 6.95-7.10 (2H, m, H-3', H-5'), 6.89 (1H, dt, J 1, 8.5 Hz, H-4').

$^{13}$C NMR (100 MHz, DMSO-d$_6$) δ 117.8 (C-3'), 117.8 (C-3), 119.6 (C-5'), 124.4 (C-α), 121.4 (C-1'), 121.6 (C-5), 131.8 (C-4), 133.9 (C-6), 127.8 (C-4'), 142.5 (C-β)

2-, 2- Dihydroxychalcone (DHC)
Preparation of 2'-Hydroxy-3, 4-dimethoxychalcone (HDMC)—

2'-Hydroxy-3, 4-dimethoxychalcone was prepared by the method of Hsieh et. al. (2000). 2- hydroxyacetophenone (3.4 g, 25 mmol), 3,4-dimethoxybenzaldehyde (4.2 g, 25 mmol) and barium hydroxide octahydrate (8.15 g, 25 mmol) were dissolved in MeOH (100 mL). The reaction mixture was stirred for 4 h at room temperature, and then evaporated in vacuo. Water (100 mL) was added, mixture neutralized with 5 % NaHCO₃ (50 mL) and extracted with EtOAc. The organic layer was separated, washed with water, dried and evaporated in vacuo. The residue was eluted through a silica gel column with cyclohexane-EtOAc (4:1) to give 2'-Hydroxy-3, 4-dimethoxychalcone.

Physical from: yellow crystalline solid. Yield: 75%, m.p: 61°C

IR (KBr)v: 1594, 1694 cm⁻¹

¹H NMR (400 MHz, CD₃OD): δ 7.36 (d, J 1.8, 1H), 6.98 (d, J 9.01, 1H), 7.29 (dd, J 9.3, 1H, 1.7 Hz, 1H), 7.61 (d, J 15.58, Hz, 1H), 7.73 (d, J 15.53 Hz, 1H), 7.81 (ddd, J 1.4, 1.6, 7.8, 2H), 7.81 (ddd, J 1.7, 7.3, 1.5 Hz, 2H), 7.57-7.59 (m, 1H), 3.86 (s, 3H), 3.89 (s, 3H). m/z: 268 (M⁺, 87), 267 (54), 253 (55), 240 (15), 238 (35), 191 (70), 163 (20), 151 (18), 105 (32), 77 (100), 51 (98%)
13 C-NMR (100MHz, CDCl₃) δ 120.0 (C-1'), 163.4 (C-2'), 118.4 (C-3'), 136.0 (C-4'), 118.6 (C-5'), 129.4 (C-6'), 127.5 (C-1'), 110.2 (C-2), 149.2 (C-3), 151.7 (C-4), 111.0 (C-5), 123.5 (C-6), 117.6 (C-α), 145.5 (C-β), 55.9 (OCH₃), 193.4 (C=O)

![2'-Hydroxy-3, 4-dimethoxychalcone](image)

**Preparation of 4', 4 – Dichlorochalcone (DCC)**

4', 4 – Dichlorochalcone was prepared following a published but slightly modified procedure (Davey and Gwilt, 1957) by condensing p-chloroacetophenone, with p- chloro-benzylidene diacetate (both prepared according to reported procedures of Vogel, 1978) in equimolar ratio in the presence of 2.5 equivalents of sodium hydroxide. A mixture of p-chlorobenzylidene diacetate (6.5g, 0.0268 mol) dissolved in (21 mL) ethanol and p- chloroacetophenone (4.13 g, 0.0268) was kept on an ice bath in a 500 ml flask. To this sodium hydroxide (2.68 g, 0.0671 mol,
10%) was added drop wise with stirring. After complete addition, the reaction mixture further stirred for 3 h, then diluted with 300 ml of ice cold water and was kept in the refrigerator overnight. The precipitate formed was filtered and washed successively with water to make it neutral. It was finally washed with 10 ml of cold alcohol and the yellow solid thus obtained was crystallized from acetone-benzene to give 4', 4 – dichlorochalcone.

Physical form: light yellow crystalline needles, Yield: 6.29 g (85%), m.p. 156°C

![4', 4 – Dichlorochalcone](image)

**Preparation of Carboxymethyl thioderivative of 4', 4 Dichlorochalcone (DCCP)-**

It was prepared by the method of Levai (1991). A solution of 4', 4 – dichlorochalcone (DCC, 850 mg, 3.07 mmol) and thioglycolic acid (355
mg, 3.84 mmol) in dry benzene (25 mL) was refluxed for 2 h with stirring at 80°C on an oil bath, then cooled, washed with water and the organic layer was dried over Na₂SO₄. The light yellow solid thus obtained on evaporation of the solvent was crystallized from acetone-benzene to furnish the desired compound.

Physical form: white crystalline needles, Yield: 870 mg (76.9 %), m.p. 116°C

\[
\text{COOH} \\
\text{m/z 276/278/280 (65.97/ 44.90/ 8.51)} \\
\text{1,3-bis(4-chlorophenyl)-3(carboxy methylthio)propan-1-one (DCCP)}
\]

Structure elucidation of DCCP - It was a white needle shaped crystalline solid, m.p. 116°C, M.W. 368 and appeared light brown on exposure to I₂ vapours (TLC). The structure of this compound has been established by IR, \(^1\text{H-NMR}\) and \(^{13}\text{C-NMR}\) spectra.

IR (KBR)ν: 3050, 825 cm\(^{-1}\).
\[ \text{\(^1\)H-NMR (400 MHz, CDCl}_3\) } \delta: 8.16 (d, 2H, H-Ar'-2,6, J 8.85 Hz); (d, 2H, H-Ar'-3,5, J 8.85 Hz); 7.32 (d, 2H, H-Ar-2,6, J 8.55 Hz); 7.50 (d, 2H, H-Ar-3,5, J 8.55 Hz) 3.68 (d, 2H, H-2, J 7.63 Hz); 4.59 (d, 1H, H-3, J 7.63 Hz ); 3.32 (d, 1H, H-l'\_up, J 15.32 Hz); 3.50 (d, 1H, H-l'\_dn, J 15.32 Hz) \\
\text{\(^{13}\)C-NMR (75 MHz, CDCl}_3\) } \delta: 45.51 (C-2), 44.54 (C-3), 196.32 (C-1), 36.17 ( C-1'), 171.02 (C-2'), 142.46 (C-Ar-1), 129.19 (C-Ar-2,6), 129.69 (C-Ar, 3,5), 139.46 (C-Ar-4), 136.55 (C-Ar'-1), 130.74 (C-Ar'-2,6), 130.61 (C-Ar'-3,5), 133.06 (C-Ar'-4) \\

**Preparation of 4'-Chloro-4-methoxychalcone (CMC)-**

4'-Chloro-4-methoxychalcone was prepared following a published but slightly modified procedure (Davey and Gwilt, 1957) by condensing p-chloroacetophenone with p-methoxybenzaldehyde (commercially available). A mixture of p-methoxybenzaldehyde (5 g, 0.0367 mol) dissolved in ethanol (15 ml) and p-cloroacetophenone (5.66 g, 0.0367 mol) was kept on an ice bath and to this sodium hydroxide (3.68 g, 0.0919 mol, 10%) was added drop wise with stirring. It was further stirred for 5h, then diluted with 300 ml of ice cold water and was kept in the refrigerator overnight. The precipitate formed was filtered and
washed successively with water to make it neutral. It was finally washed with 10 ml of cold alcohol and the yellow solid thus obtained was recrystallized from acetone-benzene to give 4’- chloro-4-methoxychalcone.

Physical form: yellow crystalline needles, Yield: 6.20 g (62 %), m.p. 121-122° C

4’- chloro-4-methoxychalcone

Preparation of Carboxymethyl thioderivative of 4’-Chloro-4-methoxychalcone (CMC)

It was prepared by the method of Levai (1991). A mixture of 4’-chloro-4-methoxychalcone (CMC, 900 mg, 3.30 mmol), thioglycolic acid (759 mg, 8.25 mmol) in dry benzene was refluxed for 5h with stirring at 80° C on an oil bath. After completion, the reaction mixture was worked up, which on crystallization from acetone-benzene yielded the above desired compound.
Physical form: yellow crystalline needles  Yield: 55 % yield, m.p: 96° C

Structure elucidation of compound-  It was in the form of yellow crystalline needles, solid, m.p. 96° C and appeared brownish-grey on exposure to I₂ vapours (TLC). The structure of this compound has been established by IR, and ¹H-NMR spectra.

IR (KBR)v: 3050, 800 cm⁻¹.

¹H-NMR (300 MHz, CDCl₃) δ: 7.83 (d, 2H, H-Ar'-2, 6, J 8.55 Hz); 7.40 (d, 2H, H-Ar'-3,5, J 8.55 Hz); 7.32 (d, 2H, H-Ar-2, 6, J 8.70 Hz); 6.83 (d, 2H, H-Ar-3,5 J 8.70 Hz); 3.00 (d, IH, H-l'^p, J 15.40); 3.11 (d, IH, H-l'^p, J 15.40); 3.52 (d, 2H, H-2, J 7.15 Hz); 4.71 (t, 1H, H-3, J 7.15 Hz); 3.77 (s, 3H, OCH₃ protons)
Figure 1 Chemical structures of the chalcones used in the study

2',4',4'- Trihydroxychalcone
Isoliquiritigenin, ISL

2',4',3,4'- Tetrahydroxychalcone
Butein, BUT

2',2'- Dihydroxychalcone
DHC

2'- Hydroxy-3,4'- Dimethoxychalcone
HDMC

4',4'- Dichlorochalcone
DCC

1,3- bis (4- chlorophenyl) - 3- (carboxy- methyl - thio) prop- an- 1- one, DCCP

4'- Chloro- 4'- methoxychalcone, CMC

1- (4- Chlorophenyl)- 3 (4- methoxy- phenyl)- 3- (carboxymethylthio) prop- an-1- one, CMCP