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Activation of Nrf2 signaling pathway by natural and synthetic chalcones: a therapeutic road map for oxidative stress

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Abstract

Introduction:

Nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway plays a key role in diverse gene expressions responsible for protection against oxidative stress and xenobiotics. Chalcones with a common chemical scaffold of 1,3-diaryl-2-propen-1-one, are abundantly present in nature with a wide variety of pharmacological properties. This review will discuss the interactions of natural and synthetic chalcones with Nrf2 signaling.

Areas covered:

Chalcones are reportedly found to activate Nrf2 signaling pathway, expression of Nrf2-regulated antioxidant genes, induce cytoprotective proteins and upregulate multidrug resistance-associated proteins. Chalcones being soft electrophiles are less prone to hostile off-target effects and unlikely to induce carcinogenicity and mutagenicity. Furthermore, their low toxicity, structural diversity, feasibility in structural reorganization and the presence of α,β-unsaturated carbonyl group which makes them suitable drug candidates targeting Nrf2-dependent diseases.

Expert opinion:

Nrf2-Keap1 signaling pathway plays a central role in redox signaling. However, available therapeutic agents for Nrf2 activation have limited practical applications due to their associated risks, relatively low efficacy and bioavailability. The designing and fabrication of new chemical entities with chalcone scaffold-based Michael acceptor mechanism should be aimed as potential therapeutic Nrf2 activators to target oxidative stress and inflammation-mediated diseases such as atherosclerosis, Alzheimer’s disease, Parkinson’s disease and many more.

Keywords: Antioxidant; Nrf2; Chalcone; Keap1; Michael acceptor mechanism
**Article highlights**

- Nrf2-Keap1 signaling pathway is a key driver in redox signaling and inflammation, therefore, a correct approach is needed in the evaluation of the chemical entities as Nrf2 modulators.
- Despite the advancement in research focused on inflammation, most compounds are still in clinical trials and only dimethyl fumarate is currently approved as Nrf2 activator in multiple sclerosis.
- Chalcone scaffold (1,2-diphenyl-2-propen-1-one), abundantly present in nature, have shown promising results for Nrf2 activation.
- Chalcones act as Michael acceptors and readily reacts with the cysteine residues of proteins.
- In this paper, we reviewed the mechanisms and structure activity relationships of chalcones as potential therapeutic agents targeting Nrf2-dependent diseases.
- Our review will improve the understanding for designing and synthesis of novel compounds with chalcone scaffold as Nrf2 activators.
1. Introduction

Despite the evident progress made in recent years, the fight against oxidative stress mediated diseases remains a great challenge [1]. The quest for the discovery of more potent antioxidant drugs has attracted huge scientific research and technological transformation in recent times. Since the first isolation of nuclear factor erythroid 2-related factor 2 (Nrf2) via cloning experiment in 1994 [2], significant advances have been made in understanding the effect of Nrf2 activation and its roles in the activation of the antioxidant response element (ARE) signaling pathway, regulation of the antioxidant and cellular protective genes, homeostatic response and especially as a pharmacological target [3-6]. Nrf2, being a basic leucine zipper region transcription factor belonging to the Cap n’ Collar family with a content of about 589 amino acid residues and the seven domains (Neh1-Neh7) has a well-developed adaptive mechanism to nullify the harmful effect of oxidative stress in biological systems [2]. Nrf2 encoded by nuclear factor erythroid-derived 2-like 2 (NFE2L2) gene plays a regulatory role in the antioxidant protein expression that inhibits oxidative stress and related consequences such as inflammation in many diseases [2,7]. Nrf2 signaling pathway is also central to the complex regulatory network of the human body and plays some indispensable roles in biological processes such as inflammation, proteostasis, metabolism, immune response and autophagy [8,9].

Several antioxidants have emerged as possible Nrf2 pathway activators amongst which chalcones exhibit excellent activity having several advantages over others [10-14]. Chalcone scaffold (1,2-diphenyl-2-propen-1-one) commonly called chalconoid (Figure 1), a privileged molecule of medicinal importance, is a Michael acceptor and a precursor for the flavonoid family with therapeutic potential against several diseases [15-16]. Structurally, chalcone is made up of aromatic rings linked by α, β-unsaturated ketone group that is linear or nearly planar in structure and this structural uniqueness accounts for its multiple biological activities [17-18]. This structure also helps chalcones to have unrestricted interactions with several proteins associated with cell apoptosis and proliferation [19-20]. Moreover, it also consists of conjugated double bonds and a delocalized π-electron system on the two aromatic rings [21]. Chalcones exhibit a broad spectrum of biological properties [22] such as antioxidant [23], anticancer [24], anti-inflammatory [25-26], antifungal [27], antimalarial [28], antitumor [29], antiprotozoal [30-31] activities. However, the effect of chalcones on Nrf2 signaling pathway activation and their recent updates need to be reviewed due to the importance of Nrf2 activation as a therapeutic target in several pathological processes mediated by oxidative stress.
The mechanism of the protective activity exhibited by phase II detoxification and antioxidant enzymes in the prevention of inflammation, carcinogenesis and oxidative stress mediated diseases is governed by Nrf2 signaling pathway [14,32]. Nrf2 plays a key regulatory role over antioxidant gene expressions and metabolic pathways in different cell lines [33-36]. Consequently, the Nrf2 activation is proposed to be a potent therapeutic target for metabolic, autoimmune, neurodegenerative, cardiovascular diseases and other prevalent diseases in which oxidative stress has been implicated [37-40]. In this regard, the activities of several natural and synthetic ARE gene inducers and Nrf2 activators such as dithiolethione, sulforaphane, oltipraz, CDDO, auranofin, curcumin and CAPE have been extensively studied [41]. Nevertheless, the functions of chalcones as potential Nrf2 pathway activator even though they are known α, β-unsaturated ketone-bearing Michael acceptors and Keap1 cysteine thiol modifiers, remain unexplored [15-16,23]. Thus, in this review we will exploit the antioxidant activity of chalcones in Nrf2 pathway activation and we will summarize several aspects of naturally occurring chalcones, chalcone synthesis and their synthetic modifications to provide valuable information for the future designing and development of new chalcone-based Nrf2 activators.

2. Nrf2-Keap1 signaling pathway as a therapeutic target in multiple diseases

Nrf2 activation has attracted significant research interest as a therapeutic target for several chronic diseases. Its biological impact can be measured primarily by its transcriptional activities and intracellular localization [42]. Nrf2 and its principal negative regulator, the E3 ligase adaptor Kelch-like ECH-associated protein 1 (Keap1), plays a multi-faceted role in several biological processes such as antioxidant metabolism, prevention of xenobiotic and endobiotic related oxidative damage [43], redox signaling, protection against triptolide-induced oxidative stress [44], metabolism of carbohydrates [45], lipids and iron [46], and inflammation regulation [47]. Nrf2 activation could lead to the induction of several cytoprotective genes as listed below.

i. Nrf2-dependent NAD(P)H quinone oxidoreductase I (NQO1) catalyze the reduction of reactive Quinones and a range of other organic compounds, responsible for redox cycling and oxidative stress [48-49]. NQO1 is responsible for reduction of free radicals and detoxification of xenobiotics and related toxicities [50].

ii. Sulfiredoxin I (Srxn1) and thioredoxin reductase I (TrxRI) act via Nrf2-dependent pathway for the reduction of ROS including hydrogen peroxide and other peroxides [51-52].
iii. Nrf2-dependent heme oxygenase-1 (HO-1) [53] is responsible for the catalytic breakdown of heme into biliverdin which is further converted into bilirubin and, thereby play a role in oxidative stress and inflammation in hypertension [54], sepsis [55], atherosclerosis [56] and many other diseases [57].

iv. The UDP-glucuronosyltransferase (UGT) is responsible for the catalytic conjugation of glucuronic acid moiety to various drugs, mutagens and xenobiotics in Nrf2-dependent pathway [58]. UGT plays a crucial role in drug metabolism and mediates drug resistance in several disease including cancer [59]. Deficiency of UGT causes a condition known as a gray baby syndrome [60].

v. Nrf2 also regulates multidrug resistance-associated proteins (MRPs; MRP1-MRP9), which are the major transporters responsible for multidrug resistance in tumor cells [61]. MRPs are also responsible for the effluxing of compounds from organs to bile and plasma for easy excretion [62].

Nrf2 activation also affects proteostasis by promoting the cleanup of oxidized or damaged proteins via protein degradation and autophagy [63]. Autophagy being an antioxidant feedback response is activated by Nrf2 via Keap1/Nrf2/p62 feedback loop and it helps to prevent intervertebral disc damage [64].

The activation of Nrf2 pathway was found to stimulate mitochondrial biogenesis via nuclear respiratory factor-1 (NRF-1) activation in cardiomyocytes [65]. Nrf2 is also responsible for the modulation of inflammation via suppression of pro-inflammatory genes and redox homeostasis [66]. In adipose tissue, Nrf2 is involved in the adipogenic differentiation of mesenchymal stem cells [67]. Furthermore, Nrf2 was found to regulate intermediary metabolism, such as, serine biosynthesis [68]. It also maintains the expansion of the stem and progenitor cells via positive regulation of chemokine receptor type 4 (Cxcr4) [69].

Nrf2 deficiency or depletion has also been linked to prolonged negative biological responses such as undue and protracted inflammatory and fibrotic responses [70]. Zhang and co-workers [71] reported the effect of depletion of Nrf2 on isoliquiritigenin protective activity against pancreatic injury and intestinal dysfunction. Nrf2 depletion resulted in the aggravation of severe acute pancreatitis (SAP)-induced damage and attenuation of isoliquiritigenin (ISL) inhibitory effect on intestinal tissue and pancreatic damage [71]. ISL induced elevation of the NF-kB and reduction of IkB protein expression in the tissue of the pancreas and intestine after SAP [71]. Considering the effects of Nrf2 activation and depletion
on essential biological processes, Nrf2 pathway becomes an important pharmacological target worthy of in-depth study and medicinal exploration.

3. Structure and properties of Chalcones

The structural uniqueness of chalcone is its open C-ring with a linear chain of three carbon atoms that connects rings A and B [72] as represented in Figure 2. Chalcone-based scaffold is an aromatic ketone and an enone with physical properties as mentioned in Table 1 [73]. Interestingly, the potential antioxidant activity of chalcones is dependent on the structure of the two aryl rings, and their substitution patterns [74].

It has been reported that, free hydroxyl group at C-2\textsuperscript{i} in ring A, a catechol group in ring B and the linking α,β-double bond are responsible for high antioxidant and other pharmacological activities of chalcones [75-77]. The presence of a catechol moiety has been linked to high radical scavenging activity in phenolic compounds and absence of one of the catechol OH groups weaken the radical scavenging ability of polyphenols [78-79]. Stepanic et al [76] reported that some alkyl substituted pyrazine derivatives of chalcones with monomethylated catechol group in the ring B showed high DPPH radical scavenging activity and their antioxidant mechanism complied with the two-step single electron transfer followed by proton transfer pathway (SET-PT) based on the quantum chemical modeling. Similarly, Vasquez-Martinez et al [77] reported that the antioxidant activity of polyoxygenated chalcones increased significantly with the presence of the catechol group when compared with derivatives without the catechol moiety. However, they observed that –OH group in \textit{meta} position and –OCH\textsubscript{3} group in the \textit{meta} and \textit{ortho} position in ring A, –OCH\textsubscript{3} group at position 3 of ring B diminished the antioxidant activities of polyoxygenated chalcones [77]. Singh et al [80] reported that, –OH group on ring A and –SCH\textsubscript{3} and –OCH\textsubscript{3} groups in the \textit{para} position of ring B play a crucial role in potent antioxidant activity. Mojarrab et al [81] reported that a methoxy group at the \textit{meta} position exerted high antioxidant activity in pyridine based chalcones in addition to \textit{ortho} substitution of the hydroxyl and fluorine moieties when compared with quercetin.

Generally, electron-donating substituents on the benzene ring have been found to favor antioxidant activity and acylation which further improves the antioxidant capacity of 4-hydroxy chalcones [3]. Kumar et al [14] in a structure-activity relationship study, reported that chalcones without any substitution on ring B lack the ability to induce Nrf2 activation, whereas chalcones with CF\textsubscript{3} substitution on ring B display significant Nrf2 activation. They reported that although \textit{ortho} CF\textsubscript{3}– substituted chalcones on ring B were the most active and non-cytotoxic, however, -
NO$_2$ at ortho position on ring B decreased Nrf2 activity and elevated toxicity significantly. Wang et al [82] reported that in contrast to chalcones with 3,4-dihydroxy groups on ring B, and with 3,4-dihydroxy groups on ring A displayed higher antioxidant activity and significant cyto-protection. They concluded that chalcones with 3,4-dihydroxyl groups on ring A exhibited significant neuroprotective activity directly through free radical scavenging and indirectly through the Nrf2 pathway activation [82].

4. Modulation mechanism of Nrf2 signaling pathway by Chalcones

Several natural and synthetic chalcones have been shown to exhibit a significant stimulatory effect on Nrf2 activity [14] and the activation of the Nrf2 pathway by chalcones was found to be a therapeutic strategy for the treatment of fibrosis in systemic sclerosis [83]. Johnson et al [83] calculated the Nrf2 pathway score using the approach of Feng et al [84] and concluded that the treatment of wild type mice with Nrf2 agonist known as 2-trifluoromethyl-2I-methoxychalcone inhibited TGF-$\beta$-induced dermal fibrosis. In another study, Kumar et al [14] measured the activation of Nrf2 by chalcones via the expression of Nrf2 dependent antioxidant genes, HO1, NQO1 and GCLM in mammalian lung epithelial cells [85]. Furthermore, they also measured in vivo the rate of Nrf2 activation by chalcones using a mouse model and also analyzed the expressions of Nfr2-regulated genes (NQO1, GCLM) in the tissue by qRT-PCR. Another report discovered a chalcone derivative 2-trifluoromethyl-2I-methoxychalcone able to increase 3-fold and 5-fold the expression of GCLM and NQO1 respectively in the small intestine comparable to sulforaphane [14].

The mechanisms of electrophilic activators including chalcones are based on the modification of Keap1 cysteine residues while the non-electrophilic activators are based on the protein-protein interaction interface of Keap1/Nrf2 [86,73].

Under normal homeostatic conditions, cytosolic Keap1 homodimerizes with the N-terminal BTB domain and binds to the cullin-based (Cul3) E3 ligase, forming Keap1-Cul3-RBX1 (Ring box protein-1) E3 ligase complex followed by ubiquitination and degradation of Nrf2 through the ubiquitin proteasome system as shown in Figure 3 [23].

The dissociation of the Nrf2-keap1 complex is essentially required for the activation of Nrf2/ARE. It is accomplished by the modification of cysteine and phosphorylation by protein kinase that results in the formation of keap1-mediated complex. The dissociation of the Keap1-Nrf2 complex and the nuclear
translocation of Nrf2 where it binds to the antioxidant response elements (ARE) leading to the transcription of ARE-driven genes as seen in Figure 3 [23].

The mechanism is facilitated by the ease of interaction of chalcones with Keap1 (protein) cysteine residues. The α,β-unsaturated carbonyl moiety of the chalcones readily forms covalent bonds with the sulfhydryl group of cysteine residues found in proteins, and this results in the formation of the Michael adduct which participates in several biological activities [21] as shown in Figure 3.

The mechanisms of Nrf2 activation by chalcones have been studied by several research groups in different stress models. Kumar et al [14] observed that the activation of Nrf2 by a chalcone derivative, (E)-1-(2-methoxyphenyl)-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one is independent of ROS or redox changes after studying whether the chalcone derivative activates Nrf2 by the generation of ROS or redox changes. They confirmed that the chalcone derivative potentially increased the expression of Nrf2-regulated antioxidant genes in the presence of N-acetyl-cysteine (NAC) and therefore suggested further studies to determine if the chalcone derivative could activate Nrf2 by direct thiol modification of Keap1. Wang et al [82] suggested that a chalcone derivative (E)-1-(3,4-dihydroxyphenyl-3-(2,5-dimethoxyphenyl)prop-2-en-I-one activates Nrf2 via promotion of Nrf2 translocation into the nucleus and induction of GCLC and HO-expression. The large number of stimuli that activate Nrf2 in addition to several cellular processes that it controls, it indicates that the mechanism of regulation of Nrf2 activity is multifactorial and intricate. The activation of Nrf2 can be modulated at the transcriptional and post-transcriptional levels by regulating the stability of protein, availability of binding partners and post-transcriptional modifications [4].

Several chalcones including both synthetic and natural, have shown the ability to modulate the Nrf2 pathway principally as activators [23]. However, a detailed research on the ability of chalcones to activate Nrf2 pathway is required for the future clinical development of chalcone derivatives. A direct or indirect interaction of natural and synthetic chalcones with Nrf2 pathway and their pharmacological effect proposes to be a sure road map to drug discovery.

5. Natural chalcones as Nrf2 inducers

Natural chalcones refer to a group of secondary metabolites widely present in spices, vegetables, tea and other medicinal plants. They are obligate intermediates in flavonoid biosynthesis with significant antioxidant properties [87-88]. Wang et al [89] reported several antioxidant natural chalcones, such as isoliquiritigenin, isobavachalcone, echinatrin, licochalcone A,B,C,D, paratocarpin B. Glypallichalcone and isoliquiritigenin were found to attenuate atherosclerosis[90],
and reduce pancreatic and intestinal damage by mitigating the oxidative stress response in mice [91-92], through Nrf2 signaling [71]. Licochalcones A, B, D, and isobavachalcone exhibited inhibitory effects on NADPH-induced microsomal lipid peroxidation [93-94]. Licochalcone A also increases antioxidant response elements in human hepatoma cells, thereby making it a possible Nrf2 pathway regulator [95]. Liu et al [96] reported that Licochalcone B exhibited stronger Nrf2 activity than TBHQ. Kim and co-workers [97] reported that licochalcone E activates Nrf2/ARE signaling pathway in microglial and neuronal cells. Gao et al [98] reported that sephadex-induced lung injury was attenuated by isobavachalcone through the activation of A20 and Nrf2/HO-1 in rats. The activity of natural chalcone derivatives on Nrf2 pathway activation is represented in Table 2.

Natural chalcones have shown significant Nrf2 activation in many studies. Miranda-Sapla et al [99] reported that trans-chalcone (1) a common precursor of flavonoids upregulates Nrf2, HO-1 and ferritin thereby modulating Leishmania amazonensis infection. Compound 1 was found to upregulate the expression of Nrf2 and ferritin, which caused a depletion of iron required for the replication and survival of the parasite [99]. This biological action indicates compound 1 a possible antileishmanial drug. Su et al [100] reported that licochalcone A (2) obtained from Glycyrrhiza radix effectively activated the Keap-Nrf2 signaling pathway and inhibited cell proliferation, apoptosis, secretion of pro-inflammatory cytokine and consequently, elevated the expression of antioxidant enzymes that determined the suppression of arthritis. They concluded that the use of compound 2 to activate Keap1-Nrf2 signaling by P62 phosphorylation at ser349 makes it a potent arthritis drug.

Kuhnl et al [101] in another study reported that compound 2 activates Nrf2, upregulates the expression of anti-inflammatory and cytoprotective enzymes, and also attenuates cutaneous oxidative stress in vivo. Lv et al [102] reported that compound 2 mediated Nrf2 activation via enhanced ARE activation and elevated Keap1 degradation in RAW 264.7 cells. In another study, Lv et al [103] reported that compound 2 activates Nrf2 signaling and enhances autophagy. They observed that the treatment of mice with autophagy inhibitor (3-methyladenine) did not inhibit the hepatoprotective effect of compound 2 due to its ability to activate Nrf2 pathway and thus, the hepatoprotective activity of compound 2 against lipopolysaccharide/d-galactosamine-induced hepatotoxicity might be mediated by its ability to activate Nrf2 and autophagy.

A recent paper demonstrated that flavokawain A (3) increased the expression of antioxidant proteins in primary splenocytes [104]. Similarly, Hseu et al [105] corroborated that compound 3 activated Nrf2/ARE signaling and induced the
expression of antioxidant proteins in A7r5 cells. They concluded that the anti-fibrotic and antioxidant activities of compound 3 due to Nrf2 activation positioned it as a potential food-based chemo-preventive drug for fibrotic ailments.

Cao et al [44] reported that a natural chalcone isoliquiritigenin (4) acts as a potent Nrf2 pathway inducer, as it activates Nrf2-associated HO-1, NQO1, and MRP2 in HepG2 cells and also exhibits significant antioxidant activity against triptolide-induced hepatotoxicity. Liu et al [106] reported that isoliquiritin (5) a natural chalcone isolated from Glycyrrhiza uralensis, activates Nrf2 antioxidant pathway, an also showed renoprotective effects in experimental membranous glomerulonephritis. It was observed that compound 5 decreased renal impairment in cationic bovine serum albumin-induced membranous glomerulonephritis in rats via Nrf2 and NF-κB pathway activation and therefore could be recommended as a potential drug candidate against renal diseases.

6. Chalcone-derived hybrids as Nrf2 activator

Synthetic chalcones have also been reported display a wide range of pharmacological activities, including activation of Nrf2 with the ability to inhibit oxidation processes and cell damage triggered by free radicals [71,107]. Consequently, these pharmacological activities of chalcones have been attributed to their antioxidant potential [74]. The antioxidant property of chalcone derivatives could be related to their ability to activate Nrf2 signaling pathway [3, 106]. So far several synthetic methods have been reported for chalcone synthesis and Claisen-Schmidt condensation under homogenous conditions in the presence of base or acid was the most studied [108-109]. Conventionally, Lewis acids and strong alkaline media have been preferred for the synthetic processes [110-112]. Modified synthetic strategies such as solvent free conditions [113], ultrasound irradiation [114], the grinding technique [115] and microwave assisted methods [116] have also been utilized.

6.1. Synthesis via Claisen–Schmidt Condensation

Claisen-Schmidt synthesis involves the condensation reaction between acetophenone and benzaldehyde derivatives, yielding unsaturated ketone including chalcones [117]. This reaction affords α,β-unsaturated aldehyde or ketone in the presence of a base or an acid [117]. Sulpizio et al [108] reported the synthesis of polyhydroxy-2-aminochalcone (6e) using compounds 6a and 6b, 6c and 6d via direct direct Claisen-Schmidt condensation in the presence of base and acid. Compound 6e (IC₅₀: 4.9µM) displayed better antioxidant activity than catechol (IC₅₀: 5.3µM) using the DPPH assay [108].
Scheme 1: synthesis of polyhydroxy-2-aminochalcone

In another study, Claisen–Schmidt condensation was used to synthesize a pyrazolic chalcone, 2-(5-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-Pyrazol-3-yl)-5-methoxyphenol (11) using compounds 7, 8, 9 and 10, this compound exhibited comparable significant antioxidant activity (89.64%) similar to Vitamin C (97.92%) based on DPPH assay [118].

Scheme 2: Synthesis of pyrazolic chalcone
Another antioxidant chalcone \( (14) \) was synthesized by Sen et al [119] via Claisen-Schmidt condensation reaction using compounds \( 12 \) and \( 13 \). This compound shows high percent inhibition (75.80%) of ROS as compared with ascorbic acid (75.02%) at a concentration of 100µg/mL as depicted in DPPH assay.

Scheme 3: Synthesis of dichlorophenoxy chalcone

Wang et al [82] reported another novel chalcone-based derivative \((E)-1-(3,4\text{-dihydroxyphenyl})-3-(2,5\text{-dimethoxyphenyl})\text{prop-2-en-1-one}) \( (17) \) with a dual antioxidant mechanism obtained via Claisen-Schmidt condensation reaction with compounds \( 15 \) and \( 16 \) in the presence of acid and base. This chalcone analogue \( (17) \) significantly scavenged free radicals and protected from H\(_2\)O\(_2\)-induced oxidative damage in PC12 cells. It reportedly activates Nrf2 pathway which could be the reason for its cytoprotective and neuroprotective effects against cerebral ischemia-reperfusion injury in animal models [82].

Scheme 4: Synthesis of dihydroxy chalcone derivative with dual antioxidant mechanisms

Vasquez-Martinez et al [77] reported polyoxygenated chalcones (compounds \( 20 \) and \( 21 \)) synthesized via Claisen-Schmidt condensation reaction using compounds \( 18 \) and \( 19 \). Compounds \( 20 \) (IC\(_{50}: 11.75\mu\text{M}\)) and \( 21 \) (IC\(_{50}: 12.45\mu\text{M}\)) were found to
exhibit higher antioxidant activity as compared with trolox (IC$_{50}$: 22.54 nM) [77].

\[
\begin{align*}
\text{Scheme 5: Synthesis of polyoxygenated chalcones} \\
\text{Wu et al [3] also reported other synthetic chalcone derivatives (compounds 24 and 25) obtained by Claisen-Schmidt condensation synthesis using compounds 22 and 23. They displayed protection against oxidative stress-induced neuronal cell death via Nrf2-ARE activation.}
\end{align*}
\]

\[
\begin{align*}
\text{Scheme 6: Synthesis of acrylate chalcone derivative} \\
\text{6.2. Synthesis of Chalcone Derivatives via Aldol Condensation} \\
\text{Aldol condensation involves the synthesis of $\beta$-hydroxyketone or $\beta$-hydroxyaldehyde via the reaction of an enol or an enolate ion with a carbonyl compound [120]. Precisely, the aldol condensation of aromatic aldehyde (26a) with acetophenone (26b) in the presence of alkali represents a simple synthetic protocol for chalcone (26c) [109].}
\end{align*}
\]
Scheme 7: Synthesis of (E)-1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one

Mojarrab et al [81] reported a pyridine based chalcone derivative (29) via aldol condensation reaction using compounds 27 and 28. Compound 29 (IC₅₀: 58.85µg/mL) displayed higher antioxidant activity than quercetin (87.24µg/mL) based on the ferrous ion chelating method and comparable antioxidant activity (IC₅₀:4.82 µg/mL) with trolox (3.83 µg/mL).

Scheme 8: The synthesis of pyridine-based chalcone derivative

Kao et al [121] synthesized a 4-aminquinolinylchalcone derivative (29e) via aldol condensation reaction of compounds 29a, 29b, 29c and 29d. Compound 29e significantly increased the Nrf2 activity in HaCaT cells and was found more potent than t-BHQ.
6.3. Synthesis via Coupling Reaction

Carboxylative Heck reaction of aryl halides (30) and styrenes (31), palladium-mediated Suzuki reaction of cinnamoyl chloride (33) and phenyl boric (32) acids represent recent methods of synthesizing chalcone (34) [109].
Ahmed and co-workers [122] reported a chalcone derivative (1-(5- methyfuran-2-yl)-3-(4-nitrophenyl))prop-2-en-1-one (37) synthesized via microwave-assisted potassium hydroxide catalyzed reaction of 2-acetyl-5-methylfuran (35) and aldehyde (36). However, the percentage inhibition (13.69%) was lower than that observed with ascorbic acid (62.34%) based on DPPH assay at 100µg/mL [122].

Similarly, using microwave irradiation, Ahmed et al [123] reported the synthesis of 1-(5-chlorothiophen-2-yl)-3-(2,4-dichlorophenyl)prop-2-en-1-one (40) using compounds 38 and 39. Compound 40 exhibited higher percentage inhibition (19.87%) than ascorbic acid (16.13%) at the concentration of 25µg/mL based on DPPH assay [123].
Scheme 12: The synthesis of chlorothiophen chalcone derivative

Stepanic et al [76] reported the antioxidant activity of alkyl substituted pyrazine derivative of chalcones (41). The antioxidant activity of compound (41) (IC_{50}: 39µM) was lower than that of vitamin C (IC_{50}: 15µM). Lee et al [124] reported a novel synthetic chalcone-coumarin hybrid (42) with significant antioxidant, neuroprotective and Aβ-aggregation reduction activity by enhancing HSPBI (heat shock protein family B (small) member 1), Nrf2, CREB-dependent survival and anti-apoptotic pathways. Kang et al [125] reported that sulfonamide-chalcones (43) inhibited the activities of β-secretase (BACE1), acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), major enzymes involved in the pathogenesis of Alzheimer’s disease (AD) in which oxidative stress has been implicated [125], You et al [126] reported an aza resveratrol-chalcone derivative (44) which exhibited significant inhibitory effect of HG-induced oxidative stress by the augmentation of antioxidant Nrf2 pathway in H9C2 cells. De Spirit et al [127] reported a chalcone derivative (45) with excellent antioxidant activity which also impacted the biosynthesis of Nrf2-regulated selenoenzymes. The activity of synthetic chalcone derivatives on Nrf2 pathway activation is represented in Table 3.
Scheme 13; Alkyl substituted pyrazine chalcone (41), Chalcone-coumarin hybrid (42), sulfonamide-chalcones (43), aza resveratrol-chalcone (44) and dihydroxy chalcone derivative (45)

Wang et al [82] designed, synthesized and evaluated the potential of chalcone analog (46) against ischemic stroke and this compound showed the dual antioxidant mechanism. Compound 46 effectively activated Nrf2/ARE antioxidant pathway and cytoprotection of H$_2$O$_2$-induced oxidative damage in PC12 cells by directly scavenging reactive oxygen species (ROS). It was recommended as a potential drug candidate for anti-ischemic stroke [82].

Kumar et al [14] reported a synthetic chalcone derivative (47) as a potent activator of Nrf2 pathway. Compound 47 activated the expression of Nrf2-regulated cytoprotective genes in the mammalian lung epithelial cells and mouse models. The induction of Nrf2-regulated transcriptional target by compound 47 was higher as compared with the positive control (sulforaphane). Similarly, Wei et al [19] reported the treatment of neonatal and adult skin fibroblasts with compound 47 demonstrating that it activates the expression of Nrf2 target genes and reduces
fibrotic responses. Therefore, compound 47 could be recommended as a possible drug candidate for the treatment of fibrosis in systemic sclerosis.

Han et al [128] reported a new neohesperidin dihydrochalcone derivative (48) that exhibited significant inhibitory effect against adipogenic differentiation of human adipose derived stem cells via Nrf2 activation. They observed that compound 48 effectively activated Nrf2 thereby inducing the expression of the antioxidant enzymes mediated by HO-1 and NQO-1 expressions and consequently reduced ROS generation in adipogenic differentiation. They suggested that compound 48 could be a potential drug for the treatment of obesity. Wu et al [3] synthesized a chalcone derivative (49) that inhibited H₂O₂-induced apoptosis in PC12 cells. Compound 49 significantly activates Nrf2 pathway and therefore could be used as a candidate drug for the treatment of oxidative stress mediated diseases [3]. Compound 49 had a preconditioning effect on Nrf2-ARE activation, thereby preventing oxidative stress-induced neuronal cell death [3].

Another study by Zhao et al [129] reported a new indolyl-chalcone derivative (50) which prevented the growth of A549 lung cancer cells via the activation of the Nrf2/HO-1 axis. The viability of A549 lung cancer cells was hindered by compound 50 through the activation of apoptosis, thereby making it a potential drug in cancer therapy.

You et al [126] synthesized an aza resveratrol chalcone analog (51) which showed anti-inflammatory and antioxidant activities associated to Nrf2 activation both in vivo and in vitro. Compound 51 inhibited high glucose-induced oxidative stress and cell fibrosis, alleviated cardiac hypertrophy, and reduced diabetes-induced myocardial inflammation through Nrf2 activation and nuclear factor-Kappa B (NF-κB) nucleus entry blockage. Therefore, it could be useful for the treatment of diabetic cardiomyopathy.

Ruiz-Miyazawa et al [130] reported that hesperidin methylchalcone (HMC) (52) induces Nrf2/HO-1 mRNA expression in a murine model of gout arthritis. HMC reduces knee joint synovitis and inhibits the depletion of endogenous antioxidants, expression of gp91phox, promotes inflammation, expression and the activity of NF-κB in the knee joint after monosodium urate (MSU) exposure. HMC significantly increases the expression of Nrf2 in MSU-exposed knee joint. Similarly, Martinez and co-workers [131] reported that hesperidin methylchalcone (HMC) (52) in tropical formulation increases mRNA expression of Nrf2 and heme oxygenase-1 (HO-1) in skin exposed to ultraviolet B (UVB) irradiation. HMC also reportedly reduces free radical levels, inhibits oxidative stress and lipid peroxidation, attenuates skin edema and also maintains glutathione level of skin.
exposed to UVB irradiation [131]. Additionally, HMC and some chalcone derivatives reportedly attenuated pain in different in vivo and in vitro models [130, 132-134]. HMC significantly reduces cytokine production, NF-κB activity and exerts protective effects against pain in mice model [132]. HMC also reduces joint mechanical hyperalgesia and leukocyte migration induced by zymosan [133]. Treatment of trans-chalcone (1) reportedly increases the mRNA expression of Nrf2 and ameliorates MSU-induced pain in acute gout arthritis in mice [134].

The toxicological scrutiny of the compounds is important to drive a decision for a novel chemical entity to be used as a therapeutic drug in practical applications [135]. So far, extensive research on efficacy, safety, and pharmacokinetics profile of natural and synthetic chalcones have been reported [136]. Physicochemical potency toxicity profiles showed that chalcones followed the Lipinski rule of five (requisite parameters for druggability) with 91.91% oral absorption values [137]. Furthermore, the maximum recommended daily dose of chalcone was determined by the local weighed approach, which showed that chalcone has a high tolerated daily dose with a score of 0.489 log mg/kg/day [137]. Toxicity profiling further confirmed an LD50 value of 2.346 log mg/kg and the oral rat chronic toxicity score of 1.403 log mg/kg per day with no skin sensations [137]. Some other reports based on the criteria of OECD also confirmed no mortality, no severe toxic effects and noncarcinogenic nature, even at the highest dose of 2000 mg/kg [138]. These results suggested safe efficacy associated with administration of chalcone.

7. Chalcones as Michael Acceptors

The majority of Nrf2 activators such as Ursodiol, sulforaphane, omaveloxolone, sulforadex, curcumin, Resveratrol, tigedlubis, rapamycin, enzastaurin, terameprocol and nordihydroguaiaretic acid are in their various stages of clinical development, and, two drugs are marketed so far [41]. Dimethyl fumarate has been approved for the treatment of diseases such as psoriasis [139], multiple sclerosis [140] while oltipraz has been evaluated for the treatment of schistosomiasis [141] and cancer [142]. Dimethyl fumarate has been reported to cause liver damage, diarrhea, and nausea [140]. Similarly, Oltipraz have low efficacy, neurotoxicity, and gastrointestinal toxicity [140-143]. Some compounds such as Cpd15 (patent: WO2013/067036), Cpd16 (patent: WO2016/202253), and 3-(pyridine-3-ylsulfonyl)-5-(trifluoromethyl)-2-one (PSTC) (patent: CN105566241A) have been patented as activators of Nrf2 acting as Nrf2-Keap1 protein-protein interaction inhibitors [41].

In this context, chalcones as Michael acceptor and potent Nrf2 activators have not yet been clinically explored. Chalcones affect several pathways such as tubulin,
NF-κB, MDM2/P53, Proteasome, TRAIL/death receptors, cell cycle, AP-I, AR, STAT3, Nrf2, ER and mitochondrial mediated apoptotic pathways thereby making chalcones a suitable drug scaffold [144]. Chalcones were reported as microtubule (tubulin) inhibitors and antitumor agents against bladder carcinoma [145]. Chalcones also suppress NF-kB-mediated inflammation and cancer [146], inhibit p53-MDM2 interaction [147], and proteasome [148], and augment the antitumor activity of TRAIL [149]. These activities confirmed the chemo-preventive properties of chalcone derivatives.

Chalcones as Michael acceptors and Nrf2 inducers have also been reported as potent anticancer agents [111,144,150]. However, the role of Nrf2 in cancer therapy remains a paradox [151]. Although Nrf2 activation protects the cells against radiation and chemically induced-carcinogenesis [152-153], it was found ineffective against induced oncogenic activation in lung cancer model [154]. Johnson et al [155] reported a complete protection against aflatoxin B(1)-induced liver cancer in rats via Nrf2 in a preclinical study. Contrarily, the protective response by Nrf2 activation promotes cancer progression, drug resistance and metastasis, this phenomenon is referred to as the dark side of Nrf2 which can be counteracted by the inhibition of Nrf2 [156-157]. Lim et al [158] reported that 4-methoxychalone induced the inhibition of Nrf2/ARE activity in A549 lung cancer cells, thereby enhancing the sensitivity of tumor cells to cisplatin an anticancer drug. Zhang et al [159] reported that a chalcone derivative (S17) that induces the activation of Keap1/Nrf2 pathway selectively inhibits the growth of gastric cancer cell lines (MGC803). Cabrera et al [160] reported brompyridine chalcone derivatives as potential chemo-preventive agents because they induce Nrf2 nuclear translocation and exert protection via the expression of downstream phase II enzymes.

The anti-inflammatory activity of chalcones as a Michael acceptor has also been linked to its ability to activate Nrf2 and inhibit NF-κB [161]. Kim et al [162] reported a chalcone derivative that downregulates inflammatory mediators in BV-2 microglial cells through Nrf2 activation, thereby attenuating cognitive impairment in a scopolamine-induced mouse model. In another study, Lee et al [163] reported that xanthohumol a chalcone derivative exerts anti-inflammatory activity via Nrf2-ARE signaling and up-regulation of downstream HO-1 in BV-2 microglial cells. Lin and co-workers [164] reported that (E)-3,4-dihydroxychalcone derivative through the activation of Nrf2/HO-1 pathway and inhibition of MAPK/NF-κB pathway exerts anti-inflammatory effect after lung cancer injury. Chalcone has strong thiol alkylating activity and therefore modulate inflammatory signaling pathways and activates the transcriptional activity of Nrf2 [165]. It was reported
that the anti-inflammatory activity of several electrophilic chalcone derivatives is related to their thiol alkylating activity [165]. The introduction of electrophiles [166] such as CF₃, Br and Cl into the α-position of the α,β-unsaturated carbonyl group of chalcone activates Nrf2, inhibits NF-κB and results in improved anti-inflammatory potency of chalcone, however substitution with stronger electrophiles such as CN and NO₂ do not enhance the anti-inflammatory activity of chalcone [165]. The inactivity of CN and NO₂ substituents could be attributed to their fast reaction with intracellular glutathione, which is available in a very small concentration [167]. Therefore, for optimal therapeutic effect via Nrf2 activation, the electrophilicity of the chalcone molecule needs to be chemically fine-tuned [165] as Nrf2 is sensitive to electrophilic stress [168].

8. Conclusion

The future development of Nrf2-targeted therapeutics remains a crucial area of medicinal interest. The activation of Nrf2 signaling pathway using chalcone derivatives has been highlighted as a pharmacological approach to a systematic amelioration of oxidative stress mediated damage in several chronic diseases. This review is an update of the scientific literature about the potential of chalcone derivatives on Nrf2 activation and associated gene expressions in the multifactorial diseases. As alluded above, substantial progress has been made in the synthesis of chalcone derivatives as well as exploration of natural chalcones for Nrf2 activation due to their structural diversity, tolerance, safety profiles and pharmacokinetic properties. However, development of more predictive models and designing of clinical trials could enable the practical applications of chalcones as therapeutics in Nrf2-dependent pathway.

9. Expert opinion

Oxidative stress with disturbances in redox signaling and inflammation play a multi-factorial role in many pathological conditions and progression of chronic diseases. Nrf2/Keap1 signaling pathway plays a pivotal role in the modulation of oxidative stress and thereby maintaining homeostasis and redox balance in cells and tissues. Research on Nrf2 so far indicated that Nrf2 plays a key role in regulating the expression of almost 250 genes that contain an enhancer sequence in the promoter regulatory regions, termed as the antioxidant response element (ARE). The crucial role of the Nrf2 signaling pathway is already established in maintaining the cytosolic and mitochondrial ROS production. In addition, recent studies also indicated the central role of the Nrf2-Keap1 signaling in the glutathione redox signaling. In response to oxidative stress, Nrf2 attenuates the ROS accumulation by regulating the levels of superoxide and peroxides via the
induction of antioxidant enzymes. Another line of evidence revealed the role of Nrf2 in the antioxidant pathway by modulating the iron metabolism pathway and the expression of genes involved in iron storage, transport and metabolism in response to oxidative stress. All these evidences suggest the need to focus on the role of Nrf2 signaling pathway in oxidative stress and inflammation-related diseases, which could enable the discovery of new therapeutics based on this pathway. It is now well recognized that Nrf2 signaling pathway regulates gene expression of proteins involved in antioxidant, detoxification and anti-inflammatory mechanisms, therefore, Nrf2-keap1 axis is an essential therapeutic target against a plethora of pathological conditions. Till date, there are several studies showing different Nrf2 activators categorized as electrophiles, multi-target drugs and Nrf2-Keap1 protein-protein interaction inhibitors (PPI). Here, a major challenge is the appropriate drug metabolism and pharmacokinetics profiles with safety for administration in peripheral and central nervous system. Therefore, an improved target selectivity is needed. Dimethyl fumarate (DMF), is the only drug approved by the US Food and Drug Administration, which acts as a therapeutic agent in multiple sclerosis and inhibits inflammation via Nrf2 antioxidant pathway. A series of clinical trials are now ongoing studying the efficacy and bioavailability of synthetic Nrf2 activators.

Among pharmacological agents, many synthetic drugs such as fumaric acid esters, sulforaphane, nitro fatty acids and many other plant-derived compounds have been studied, however, subsequent studies provided conflicting results with poor absorption, metabolism and excretion properties. In this context, chalcones attracted attention as it activates Nrf2 in a wide range of multiple disease models with safe pharmacokinetic properties. Traditionally, natural and synthetic chalcones have various therapeutic applications including anticancer, anti-inflammatory, antioxidant, and antidiabetic properties. The structure-activity relationship studies already implicated the high absorption rate, and safety profiles of chalcones. In this review, we discussed the mechanism of Nrf2 activation by different chalcones and related compounds, which indicates a multi-target Michael acceptor mechanism. This expanded recognition of chalcones as a potential therapeutic scaffold which has sparked the interest of the researchers in the designing and development of Nrf2 modulators. The designing of noble therapeutic drugs based on the target selectivity in the relevant tissues, their solubility, cell penetration ability as well as metabolic stability may be a more logical approach
for testing meaningful clinical applications of Nrf2 activators which could lead to
the long-lasting pharmacodynamic effects.

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133. Rasquel-Oliveira FS, Manchope MF, Staurengo-Ferrari L, et al. Hesperidin methyl chalcone interacts with NFκB Ser276 and inhibits zymosan-


### Table 1: Physical properties of Chalcones

<table>
<thead>
<tr>
<th>Physical state</th>
<th>Solid</th>
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<tbody>
<tr>
<td>Molecular formula</td>
<td>C\textsubscript{15}H\textsubscript{12}O</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>208.25 g/mol</td>
</tr>
<tr>
<td>Melting Point</td>
<td>57.5°C</td>
</tr>
<tr>
<td>Boiling point</td>
<td>346°C</td>
</tr>
<tr>
<td>Magnetic susceptibility</td>
<td>-125.7x10\textsuperscript{-6} cm\textsuperscript{3}mol</td>
</tr>
<tr>
<td>Density</td>
<td>1.071 g/cm\textsuperscript{3}</td>
</tr>
</tbody>
</table>

### Table 2: Description of natural chalcones as Nrf2 activators

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compounds</th>
<th>Bioactive Concentration</th>
<th>Biological Activity</th>
<th>Study Model</th>
<th>Targeted Disease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trans-chalcone</td>
<td>2-12 µM</td>
<td>Nrf2 activation, antioxidant, antileishmanial</td>
<td>Mice, L. amazonensis-infected macrophages</td>
<td>Leishmaniasis</td>
<td>[87]</td>
</tr>
<tr>
<td>2</td>
<td>Licochalcone A</td>
<td>25-50 mg/kg</td>
<td>Nrf2 activation, antioxidant, anti-arthritic</td>
<td>Mice</td>
<td>Arthritis</td>
<td>[88]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9 µM</td>
<td>Antioxidant, cytoprotective, anti-inflammatory</td>
<td>Cells</td>
<td>Cutaneous oxidative stress</td>
<td>[89]</td>
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<tr>
<td>No.</td>
<td>Compound Structure</td>
<td>Concentration</td>
<td>Effect</td>
<td>Treated Cell/Model</td>
<td>Disease</td>
<td>Reference</td>
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</tr>
<tr>
<td>3</td>
<td><img src="image" alt="Compound 3" /></td>
<td>2-30 μM</td>
<td>Antioxidant, anti-inflammatory</td>
<td>Mice, Primary splenocytes</td>
<td>Inflammatory diseases</td>
<td>[92]</td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="Compound 4" /></td>
<td>5-20 μM</td>
<td>Antioxidant</td>
<td>A7r5 cells</td>
<td>Fibrotic diseases</td>
<td>[93]</td>
</tr>
<tr>
<td>5</td>
<td><img src="image" alt="Compound 5" /></td>
<td>10 mg/kg/bw/day</td>
<td>Antioxidant, anti-inflammatory</td>
<td>mice</td>
<td>Membranous glomerulonephritis</td>
<td>[94]</td>
</tr>
<tr>
<td>Entry</td>
<td>Compounds</td>
<td>Bioactive Concentration</td>
<td>Biological Activity</td>
<td>Study Model</td>
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</tr>
<tr>
<td>46</td>
<td>(E)-1-(2,4-dihydroxyphenyl)-3-(4-(((2S, 3R, 4S, 5S, 6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)phenyl)prop-2-en-1-one (Isoliquiritin)</td>
<td>10 µmol/L</td>
<td>Nrf2 activation, Antioxidant</td>
<td>Neuron-like cell line, PC12 Cells</td>
<td>Ischemic stroke</td>
<td>[70]</td>
</tr>
<tr>
<td>47</td>
<td>(E)-1-(2-methoxyphenyl)-3-(2-trifluoromethyl)phenyl)prop-2-en-1-one</td>
<td>2.5-20 µM</td>
<td>Nrf2 activation, antioxidant, cytoprotective</td>
<td>Lung epithelial cell, mce</td>
<td>Oxidative stress</td>
<td>[7]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400 mg/kg</td>
<td>Anti-fibrotic, cytoprotective</td>
<td>Systemic sclerosis patients samples</td>
<td>Fibrosis in systemic sclerosis</td>
<td>[12]</td>
</tr>
<tr>
<td>No.</td>
<td>Chemical Structure</td>
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<td>Effect</td>
<td>Cell Type</td>
<td>Disease</td>
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<tr>
<td>48</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>5-40 µM</td>
<td>Nrf2 activation, cytoprotective, antioxidant</td>
<td>Human adipose derived stem cells (hASCs)</td>
<td>Obesity</td>
<td>[116]</td>
</tr>
<tr>
<td>49</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>10 µM</td>
<td>Nrf2 activation, antioxidant</td>
<td>Neuron-like PC12 Cells</td>
<td>Oxidative stress</td>
<td>[3]</td>
</tr>
<tr>
<td>50</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>2.5 µM</td>
<td>Nrf2 activation, antioxidant, anticancer</td>
<td>Lung cancer cell line A549</td>
<td>Lung cancer</td>
<td>[117]</td>
</tr>
<tr>
<td>51</td>
<td><img src="image4" alt="Chemical Structure" /></td>
<td>5-10 µM, 5-20 mg/kg</td>
<td>Nrf2 activation, antioxidant, anti-inflammatory</td>
<td>Cardiomyocyte H9c2 cells, mice</td>
<td>Diabetic cardiomyopathy</td>
<td>[114]</td>
</tr>
<tr>
<td>52</td>
<td><img src="image" alt="Hesperidin methyl chalcone" /></td>
<td>3-30mg/kg, 100µL</td>
<td>Antioxidant, anti-inflammatory</td>
<td>Murine model, mice</td>
<td>Gout arthropies</td>
<td>130</td>
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<tr>
<td>2.5-1600µg/mL</td>
<td>Antioxidant, anti-inflammatory</td>
<td>Mouse model</td>
<td>Skin oxidative stress and inflammation</td>
<td>131</td>
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<td></td>
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</table>

**Legends of Figures**

**Figure 1**: Structural representation of chalcone backbone.

**Figure 2**: The backbone of chalcone.

**Figure 3**: Mechanism of Nrf2 signaling pathway activation by michael addition of chalcone with cysteine of protein.
Fig 3

Homeostatic condition

Stress condition/
Chalcone

Nrf2

Cytoplasm

Ubiquitination
Homeostasis

Proteasome

Nrf2 degradation

Nucleus

Anti-inflammatory,
Antioxidant,
Detoxification

ARE

Gene transcription
NQO1, GCLM, HO-1

Translocation