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RESEARCH ARTICLE

Chalcones and *Bis*-Chalcones Analogs as DPPH and ABTS Radical Scavengers

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Abstract: ***Background*:** A number of synthetic scaffolds, along with natural products, have been identified as potent antioxidants. The present study deals with the evaluation of varyingly substituted, medicinally distinct class of compounds "chalcones and *bis*-chalcones" for their antioxidant potential.

***Methods*:** *In vitro* radical scavenging activities were performed on a series of synthetic chalcones **1-13** and *bis*-chalcones **14-18**.

***Results*:** All molecules **1-18** revealed a pronounced 2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-*bis*(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radicals scavenging potential in the ranges of IC_{50s} = 0.58 ± 0.14 - 1.72 ± 0.03 and 0.49 ± 0.3 - 1.48 ± 0.06 μM, respectively. Ascorbic acid (IC_{50s} = 0.5 ± 0.1 and 0.46 ± 0.17 μM for DPPH and ABTS, respectively) was used as a standard radical scavenger.

***Conclusion*:** Structure-activity relationship (SAR) revealed an active participation of various groups, including -SMe and -OMe in scavenging activity.

Keywords: Chalcones, *bis*-chalcones, reactive oxygen species (ROS), DPPH, ABTS, *in vitro*.

1. INTRODUCTION

Free radicals (FRs) are known to be highly reactive species, possessing one or more 'unpaired' electron in their last atomic or molecular orbital. Oxygen-based FRs are highly important in biochemical processes. These oxygen FRs include superoxide (O_2^{-}), alkoxyl ($RO^{\bullet}$), peroxyl ($ROO^{\bullet}$), hydroxyl ($^{\bullet}OH$), and hydroperoxyl ($HOO^{\bullet}$) radicals. $^{\bullet}OH$ and $HOO^{\bullet}$ radicals normally result from the homolytic fission of hydrogen peroxide (H_2O_2). There are several non-radical reactive oxygen-based species of biological interest such as singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), nitric oxide (NO), and peroxynitrite (ONOO) [1, 2]. In the human body, several continuing biochemical reactions encompass through free-radical mechanisms. Oxidation, hydroxylation, and carboxylation are the biochemical processes that engage single-electron transfers from a cofactor or an FR intermediate. These FR intermediates are associated with cytokine, ion transport, metabolic energy formation, neuro-modulation, growth factor, hormone action, RNA transcription, and programmed cell death [3].

Reactive oxygen species (ROS) are produced in the human body from either exogenous or endogenous sources. Endogenously, ROS can be formed in soluble oxidase enzyme systems, microsomal and mitochondrial electron transport systems, and during phagocyte activation [4]. However, exogenously, ROS can be generated from cigarette smoke, air pollutants, water pollutants, heavy metals, radiation, organic solvents, and certain drugs, *etc*. [5]. Radical and non-radical based ROS, depending upon the concentration within the biological system, can either be toxic or advantageous.

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At low or moderate levels, ROS play beneficial roles in the activation of the host immunity system, signal transduction, and gene transcription, *etc*. [6, 7]. However, excessive formation leads to oxidative stress, which is accountable for modifying several pathological situations and aging. Accumulation of ROS in the body is very threatening as these species are harmful to the pivotal biological entities like DNA, proteins, and lipids [8-10]. These free radicals can cause several severe complications, including cardiovascular diseases, cancer, and diabetes [11]. ROS also trigger neurodegenerative disorders such as Alzheimer's and Parkinson's diseases [12].

Antioxidants are biologically active agents that are used to slow down the production or completely end up the chain reactions of free radicals [13-16]. Internal antioxidants work as a defence system to prevent the human body from oxidative damage. These internal oxidants are enzymes, including superoxide dismutase (SOD), glutathione peroxidase, catalase, and thioredoxin or naturally occurring compounds in the body, such as ascorbic acid, uric acid, lipoic acid, glutathione, and *R*-tochopherol. But once the internal antioxidants are overwhelmed, the external sources of antioxidants are needed to reduce oxidative stress [17, 18]. Butylated hydroxytoluene, lactoferrin, transferrin, and ceruloplasmin sequester transition metals are well-known synthetic antioxidants [19]. Few chemical classes of compounds based on phenols, sterically crowded amines, sulphides, and phosphorous compounds are known to possess antioxidant potential [20]. Therefore, it is immensely essential to evaluate the diverse classes of organic scaffolds for their ROS scavenging potential.

Chalcones (1,3-diaryl-2-propen-1-ones) are ubiquitously found as natural products in a variety of vegetables, fruits, tea, spices, and soy-based food stuff. They are recognized as precursors for the biosynthesis of flavonoids and isoflavonoids [21]. Flavonoids and isoflavonoids have the ability to scavenge free radicals and to stop lipid peroxidation [22]. Chalcones are chemically composed of two aromatic rings joined together by an α,β-unsaturated carbonyl system. Thus, chalcones commonly known as α,β-unsaturated ketones,

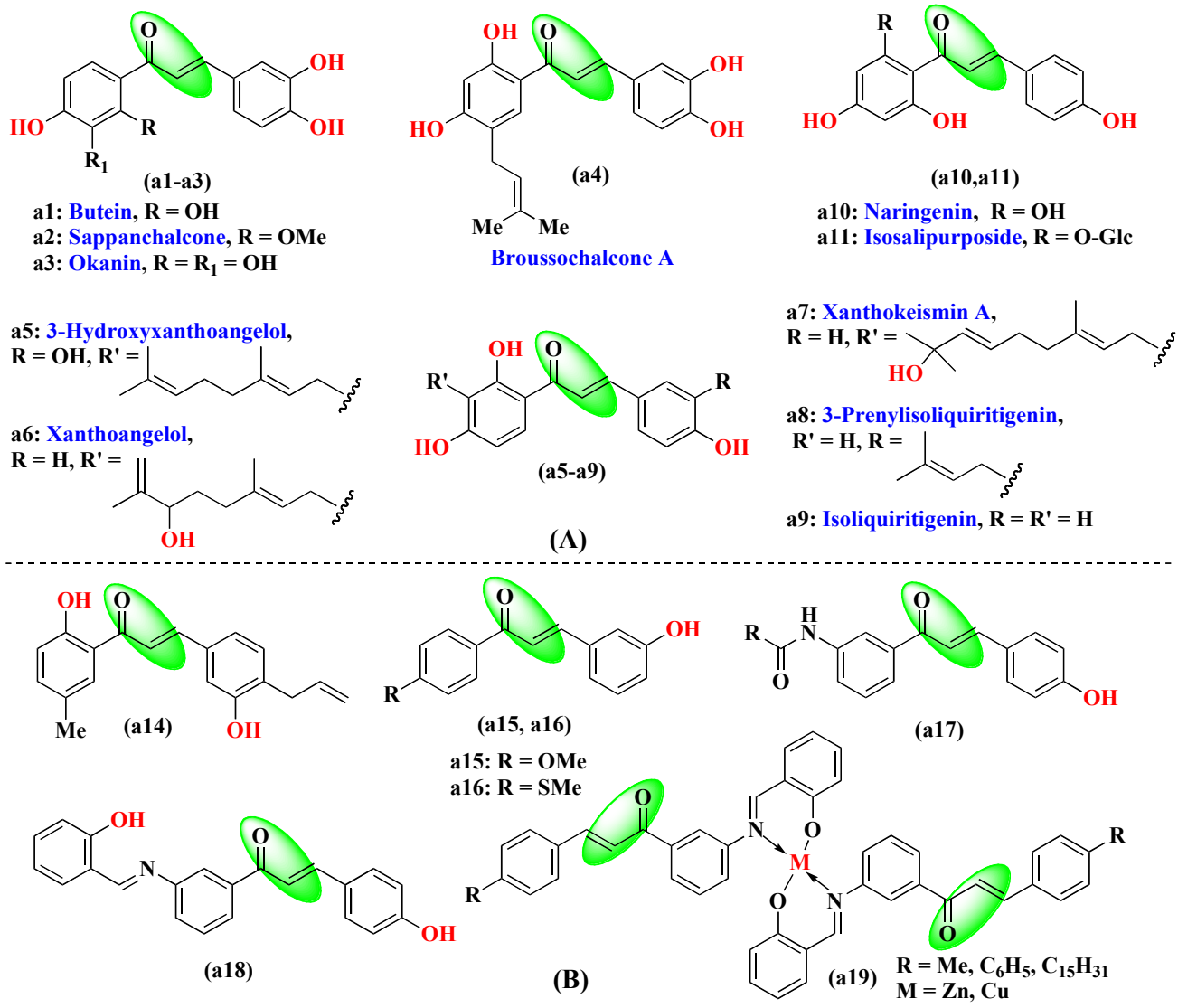


Fig. (1). Naturally occurring chalcones (**A**); Synthetic chalcones (**B**) as antioxidants.

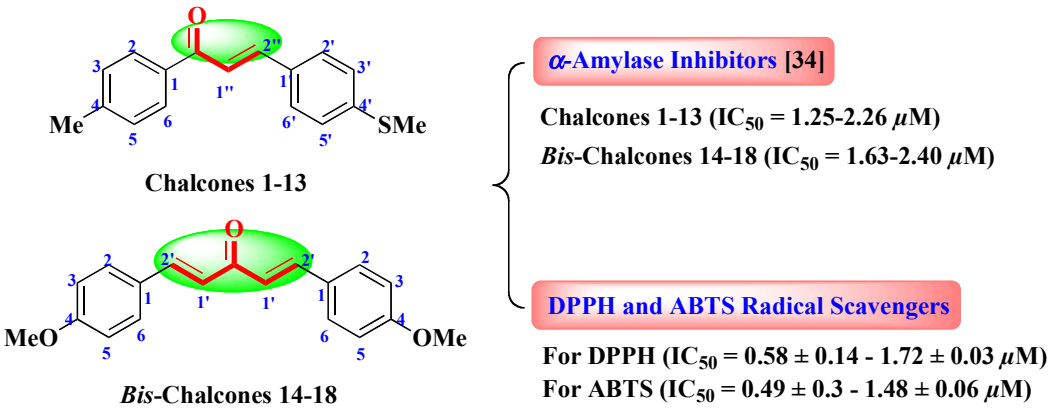


Fig. (2). Leads having hypoglycemic and antioxidant potential.

exist in *trans* and *cis* isomers, and are regarded as a significant class of natural drugs. Naturally occurring chalcones usually possess polyhydroxylated aryl rings [23, 24]. Chalcones are commonly synthesized by acid or base catalysed Claisen-Schmidt condensation reaction of aldehyde and ketone [25, 26]. *Bis*-chalcone is extensively used as a starting material and intermediate for the syntheses of valuable drugs [27, 28]. Chalcones are medicinally important scaffolds, possess noticeable and indispensable broad biological properties such as antitumor, anticancer, anti-inflammatory, antimalarial, antiprotozoal, anti-HIV, antimicrobial, and antioxidant activities [29-31].

A number of naturally occurring hydroxychalcones, including butein (**a1**), sappanchalcone (**a2**), and okanin (**a3**), are known to be effective antioxidants in the concentration range of 0.025-0.1%. However, isoprenylated chalcone such as broussochalcone A (**a4**) is known to inhibit iron-induced lipid peroxidation in rat brain homogenate in a concentration-dependent manner (IC_{50} = 0.63 ± 0.03 μM) and has also been found to suppress the NO production (IC_{50} = 11.3 μM) in LPS activated macrophages. Prenylated chalcone, 3-hydroxyxanthoangelol (**a5**) showed excellent DPPH radical scavenging potential (IC_{50} = 5.0 μg). Xanthoangelol B (**a6**), xanthokeismins A (**a7**) were found to be potent superoxide scavengers (IC_{50} = 0.5 - 1.1 μM). Furthermore, prenylated chalcone 3-prenyl isoliquiritigenin (**a8**) was identified as a good DPPH radical scavenger than non-prenylated derivative isoliquiritigenin (**a9**) [32]. In addition to that, synthetic chalcones, including allylated chalcone (**a14**) having OH and Me substitution, were identified as an excellent antioxidant (DPPH = 41%) [33]. Similarly, synthetic chalcones bearing -OH moiety on ring A and -OMe or -SMe group (**a15**, **a16**) at *para* position of ring B were identified as potent antioxidants. Moreover, zinc and copper complexes of chalcones were also reported to possess antioxidant potential (**a18**, **a19**) (Fig. **1**) [30].

Recently, our research group has reported chalcones and *bis*-chalcones **1-18** as potent α-amylase inhibitors in search of leads for the discovery of antidiabetic agents [34] (Fig. **2**). It is a well-known fact that ROS is frequently associated with hyperglycemia and diabetic complications [35-38]. Scientists have a keen interest in discovering chemical agents that must have hypoglycemic and antioxidant potentials to treat diabetes mellitus [39]. Knowing the remarkable antioxidant potential of chalcones, we intended to screen these synthetic chalcones and *bis*-chalcones based α-amylase inhibitors **1-18** for their possible radical scavenging potential against DPPH and ABTS radicals. There are numerous methods for the estimation of antioxidants potential, but researchers mostly prefer DPPH and ABTS radical scavenging assays due to the utilization of stable free radicals. These assays are also popular due to their speed, ease, and sensitivity [40-41]. This piece of research discusses the antioxidant potential of chalcones and *bis*-chalcones revealing extinction at different wavelengths, *e.g.* 517 and 734 nm in DPPH and ABTS assays, respectively, were used to observe colored radical depletion *via* spectrophotometer.

2. EXPERIMENTAL

2.1. General Procedure for the Synthesis of Chalcones and *bis*-chalcones 1-18

Detailed protocol for the synthesis of chalcones and *bis*-chalcones has been published earlier [34]. Spectral data for all compounds has been provided as supplementary information (SI).

2.2. DPPH Free Radical Scavenging Assay

The ability of the sample to scavenge DPPH free radicals was evaluated by the standard method [41, 42]. The sample solutions were prepared in absolute alcohol, ranging from 0.01 mg/mL to 1 mg/mL. A total of 500 μL of the sample was added with 500 μL of DPPH solution (2 μmol). After 20 min of incubation, the samples were placed in the dark at room temperature. Absorbance was taken at 517 nm. 500 μL of prepared DPPH solution and 500 μL of absolute alcohol were used as control. A similar procedure was repeated for ascorbic acid as standard.

The percentage inhibition of radical scavenging activity was calculated as illustrated,

% inhibition = 100*(Absorbance-DPPH control - Absorbance-sample/Absorbance-sample)

2.3. ABTS Free Radical Cation Scavenging Assay

ABTS free radical cation scavenging ability of the compounds was determined by standard method [41, 42]. 7 mM

ABTS was dissolved in distilled water and 2.45 mM potassium persulfate was added to prepare ABTS free radical cations. The solution was kept in the dark for 12-16 h at room temperature. Sample solutions were prepared in absolute alcohol ranging from 0.01 mg/mL to 1 mg/mL. The samples were added with 1 mL of ABTS solution and incubated for 30 min. Absorbance was taken at 734 nm and the procedure was repeated for ascorbic acid as standard.

The percentage inhibition of radical scavenging activity was calculated as illustrated,

% inhibition = 100*(Absorbance-ABTS control - Absorbance-sample/Absorbance-sample)

3. RESULTS AND DISCUSSION

Synthesis of chalcones and *bis*-chalcones **1-18** has been reported earlier [34]. In brief, chalcones and *bis*-chalcones **1-18** were synthesized by treating substituted acetophenones or acetone with benzaldehyde derivatives under basic condition as depicted in Scheme **1**. All compounds were fully characterized by various spectroscopic techniques (see supplementary information).

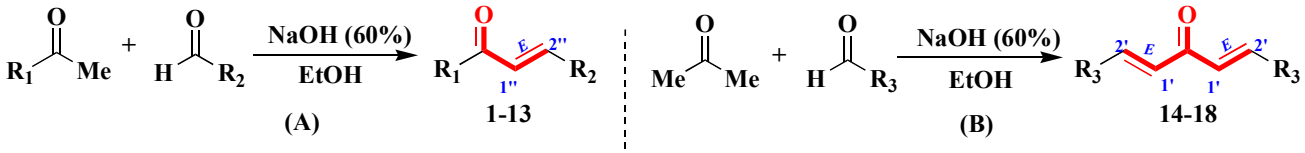
3.1. Radicals (DPPH and ABTS) Scavenging Activities of Synthetic Compounds 1-18

Diversely substituted synthetic chalcones and *bis*-chalcones **1-18** were evaluated for radical (DPPH and ABTS) scavenging activities *in vitro*. It is worth mentioning that the whole library was found to be significantly active and showed good to moderate DPPH and ABTS radical scavenging activities in the ranges of IC_{50s} = 0.58 ± 0.14 - 1.72 ± 0.03 and 0.49 ± 0.3 - 1.48 ± 0.06 μM, respectively. Activity results were compared to the standard ascorbic acid (IC_{50s} = 0.5 ± 0.1 and 0.46 ± 0.17 μM for DPPH and ABTS radical scavenging activities, respectively) (Table **1**).

3.2. Structure-activity Relationship (SAR)

SAR was anticipated on the basis of varying substitutions (R_1, R_2, and R_3) and their effects on radical scavenging potential. Amongst chalcones **1-13**, compounds **1-6** possess -Me substitution on ring R_1 and different substitutions including -OMe, -OEt, -SMe, -Cl, -OH, and -OBn on ring R_2. Compound **4** (DPPH: IC_{50} = 0.66 ± 0.1 μM) (ABTS: IC_{50} = 0.52 ± 0.2 μM) having *p*-SMe substitution, was identified as the most active radical scavenger and showed scavenging potential comparable to the standard ascorbic acid (IC_{50s} = 0.5 ± 0.1 and 0.46 ± 0.17 μM for DPPH and ABTS, respectively). Replacement of -SMe to -OMe in case of compound **1** (DPPH: IC_{50} = 1.16 ± 0.09 μM) (ABTS: IC_{50} = 1.09 ± 0.08 μM) led to two-fold decreased scavenging potential which suggests that S atom played a crucial role in scavenging radicals. Similarly, replacing -SMe with -Cl group in compound **2** (DPPH: IC_{50} = 0.94 ± 0.16 μM) (ABTS: IC_{50} = 0.74 ± 0.15 μM) resulted in slight decline in scavenging ability. Another compound **3** (DPPH: IC_{50} = 1.21 ± 0.1 μM) (ABTS: IC_{50s} = 1.09 ± 0.08 μM) having a combination of -OMe and -OEt showed scavenging strength almost similar to compound **1** which suggests that incorporation of extra -OEt did not participate in the enhancement of scavenging ability. The same was the case of compound **6** (DPPH: IC_{50} = 1.67 ± 0.05 μM) (ABTS: IC_{50} = 1.27 ± 0.04 μM) that having a combination of -OH and -OEt groups revealed a slight decline in the scavenging potential as compared to compound **3**. Hydroxy group in compound **6** might involve intramolecular hydrogen bonding with adjacent -OEt thus being less liable to stabilize the free radicals. Furthermore, *p*-OBn substituted compound **5** (DPPH: IC_{50s} = 1.1 ± 0.1 μM) (ABTS: IC_{50s} = 0.94 ± 0.1 μM) showed scavenging strength comparable to other compounds including **1-3**, and **6** (Fig. **3**).

Chalcones **7-14** having *p*-OMe group on ring R_1 and varying substitutions on second ring R_2. As observed in the

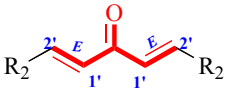


Comp.	R_1	R_2
1	*p*-Tolyl	4-Methoxyphenyl
2	*p*-Tolyl	4-Chlorophenyl
3	*p*-Tolyl	4-Ethoxy-3-methoxyphenyl
4	*p*-Tolyl	4-(Methylthio)phenyl
5	*p*-Tolyl	4-(Benzyloxy)phenyl
6	*p*-Tolyl	3-Ethoxy-2-hydroxyphenyl
7	4-Methoxyphenyl	4-Methoxyphenyl
8	4-Methoxyphenyl	2,4-Dichlorophenyl
9	4-Methoxyphenyl	4-Bromophenyl
10	4-Methoxyphenyl	4-(Methylthio)phenyl
11	4-Methoxyphenyl	4-(Benzyloxy)phenyl
12	4-Methoxyphenyl	1,1'-Biphenyl
13	4-Methoxyphenyl	4-Ethoxyphenyl

Comp.	R_3
14	4-Chlorophenyl
15	4-Bromophenyl
16	4-Methoxyphenyl
17	4-(Methylthio)phenyl
18	3-Ethoxy-2-hydroxyphenyl

Scheme 1. Synthesis of (**A**) chalcones **1-13** and (**B**) *bis*-chalcones **14-18**.

Table 1. Radical (DPPH and ABTS) scavenging activities of chalcones and *bis*-chalcones 1-18.

Comp.	DPPH $IC_{50} \pm SEM^a$ (μM)	ABTS $IC_{50} \pm SEM^a$ (μM)	Comp.	DPPH $IC_{50} \pm SEM^a$ (μM)	ABTS $IC_{50} \pm SEM^a$ (μM)
 **Chalcones**					
1	1.16 ± 0.09	1.09 ± 0.08	**8**	1.06 ± 0.1	0.87 ± 0.1
2	0.94 ± 0.16	0.74 ± 0.15	**9**	1.08 ± 0.2	0.89 ± 0.12
3	1.21 ± 0.1	1.09 ± 0.08	**10**	0.58 ± 0.14	0.49 ± 0.3
4	0.66 ± 0.1	0.52 ± 0.2	**11**	1.56 ± 0.04	1.23 ± 0.12
5	1.1 ± 0.1	0.94 ± 0.1	**12**	1.12 ± 0.04	0.98 ± 0.22
6	1.67 ± 0.05	1.27 ± 0.04	**13**	1.14 ± 0.05	1.08 ± 0.14
7	0.98 ± 0.03	0.75 ± 0.12			
 ***Bis*-Chalcones**					
14	0.81 ± 0.1	0.71 ± 0.2	**17**	1.72 ± 0.03	1.48 ± 0.06
15	0.9 ± 0.1	0.73 ± 0.1	**18**	1.46 ± 0.07	1.20 ± 0.09
16	0.76 ± 0.2	0.58 ± 0.3	Ascorbic acid[b]	0.5 ± 0.1	0.46 ± 0.17

SEM^a Standard error mean); Ascorbic acid[b] (Standard for DPPH and ABTS radical scavenging activity); Units of IC_{50} are in μM.

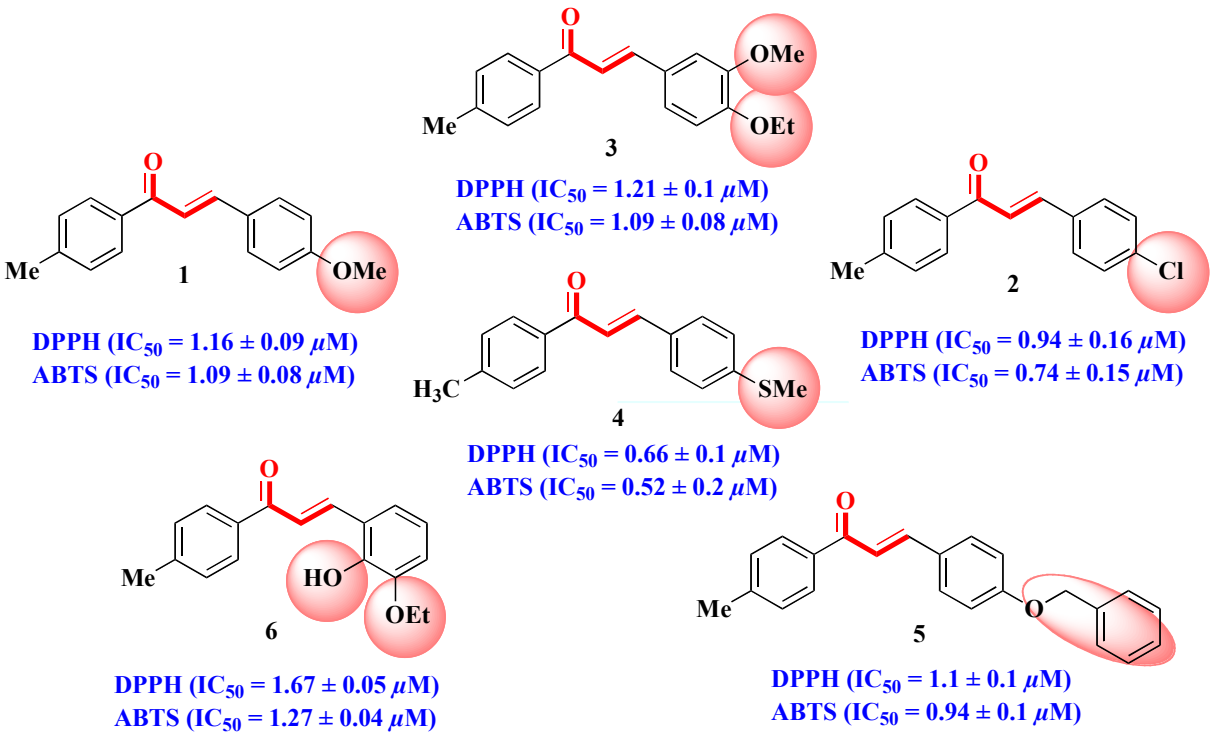


Fig. (3). SAR of compounds **1-6**.

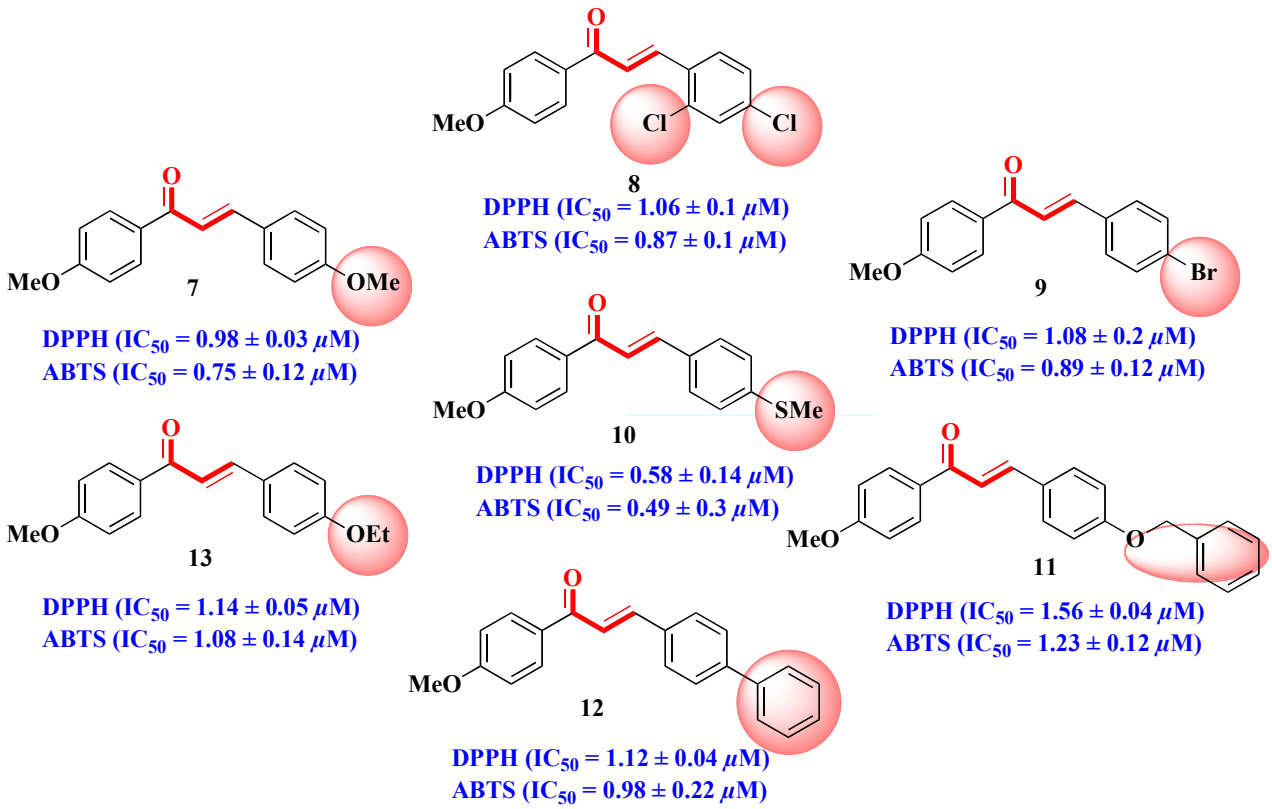


Fig. (4). SAR of compounds **7-13**.

previous case, *p*-SMe substituted compound **10** (DPPH: IC_{50} = 0.58 ± 0.14 μM) (ABTS: IC_{50} = 0.49 ± 0.3 μM) revealed significant radical scavenging strength which further confirmed the active participation of -SMe group in scavenging potential. It is worth mentioning that compound **10** showed DPPH and ABTS radical scavenging activities, almost similar to standard ascorbic acid (IC_{50} = 0.5 ± 0.1 and 0.46 ± 0.17 μM for DPPH and ABTS, respectively). Replacement of -SMe with -OMe (comp. **7**), -OEt (comp. **13**), and -Br (comp. **9**) groups showed almost two-fold decreased scavenging strength. Compound **8** (DPPH: IC_{50} = 1.06 ± 0.1 μM) (ABTS: IC_{50} = 0.87 ± 0.1 μM) with *o,p*-dichloro groups also revealed scavenging strength comparable to analogs **7**, **9**, and **13**. Compounds **11** (DPPH: IC_{50} = 1.56 ± 0.04 μM) (ABTS: IC_{50} = 1.23 ± 0.12 μM) and **12** (ABTS: IC_{50} = 1.12 ± 0.04 μM) (ABTS: IC_{50} = 0.98 ± 0.22 μM) having *p*-Ph and *p*-OBn, respectively, showed scavenging strength to be good as revealed by other compounds. For sure, extended conjugation in the case of compounds **11** and **12** played a crucial role to scavenge radicals (Fig. **4**).

Bis-chalcones **14-18** also showed good radical scavenging strength in the range of IC_{50} = 0.76 ± 0.2 - 1.72 ± 0.03 μM. In contrast to chalcones, *p*-OMe substituted residue **16** (DPPH: IC_{50} = 0.76 ± 0.2 μM) (ABTS: IC_{50} = 0.58 ± 0.3 μM) was found to be better radical scavenger than *p*-SMe substituted molecule **17** (DPPH: IC_{50} = 1.72 ± 0.03 μM) (ABTS: IC_{50} = 1.48 ± 0.06 μM). Compounds **14** (DPPH: IC_{50} = 0.81 ± 0.1 μM) (ABTS: IC_{50} = 0.71 ± 0.2 μM) and **15** (DPPH: IC_{50} = 0.9 ± 0.1 μM) (ABTS: IC_{50} = 0.73 ± 0.1 μM) having *p*-Cl and *p*-Br groups, respectively, demonstrated almost similar scavenging strength which suggests that Cl and Br groups equally participated in scavenging the DPPH and ABTS radicals. As observed in case of chalcones, *bis*-chalcone **18** (DPPH: IC_{50} = 1.46 ± 0.07 μM) (ABTS: IC_{50} = 1.20 ± 0.09 μM) bearing -OH and -OEt groups displayed moderate radical scavenging strength (Fig. **5**).

In brief, limited SAR proposed that among all varying substitutions, -SMe and -OMe groups actively participated in scavenging the DPPH and ABTS radicals.

3.3. Comparison of Current Leads with Previously Identified Radical Scavengers

During the last decade, our group has identified different classes of compounds for their potential radical scavenging activities [41-47]. Recently, we identified *N*-sulfonohydrazide substituted indazoles (**A**) as potential DPPH (IC_{50} = 2.01 - 5.3 μM) and ABTS (IC_{50} = 2.34 - 5.5 μM) radical scavengers. In this study, we recognized that sulfonohydrazide bearing molecules was found to be better scavenger as compared to indazole [43]. While in the present case, we identified -SMe bearing analogs as the best DPPH and ABTS scavengers which accredited that sulfur atom might play an important role in radical scavenging phenomena. In addition to that, hybrid hydrazinyl thiazole substituted chromones (**B**) showed remarkable DPPH (IC_{50} = 0.09-2.23 μM) and ABTS (IC_{50} = 0.584-3.738 μM) scavenging activities [41]. Similarly, hybrid hydrazinyl thiazole substituted coumarins (**C**) also showed good DPPH and ABTS radical scavenging activities in the ranges of IC_{50} = 0.62 - 1.952 μM and IC_{50} = 0.37 - 1.844 μM, respectively [42]. It is

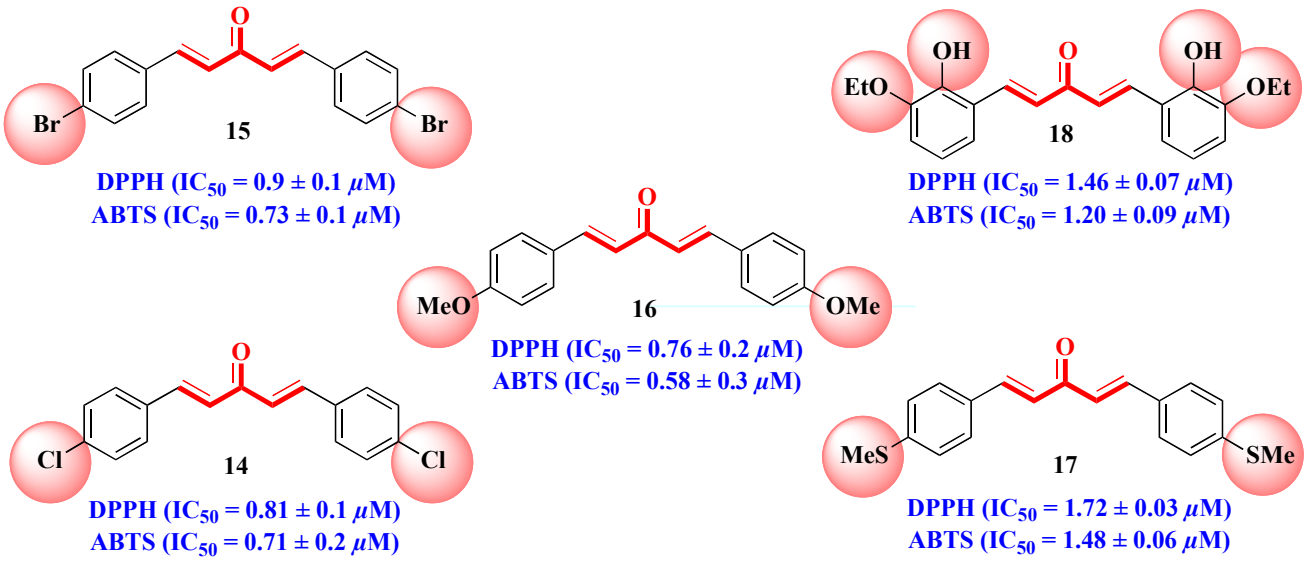


Fig. (5). SAR of compounds **14-18**.

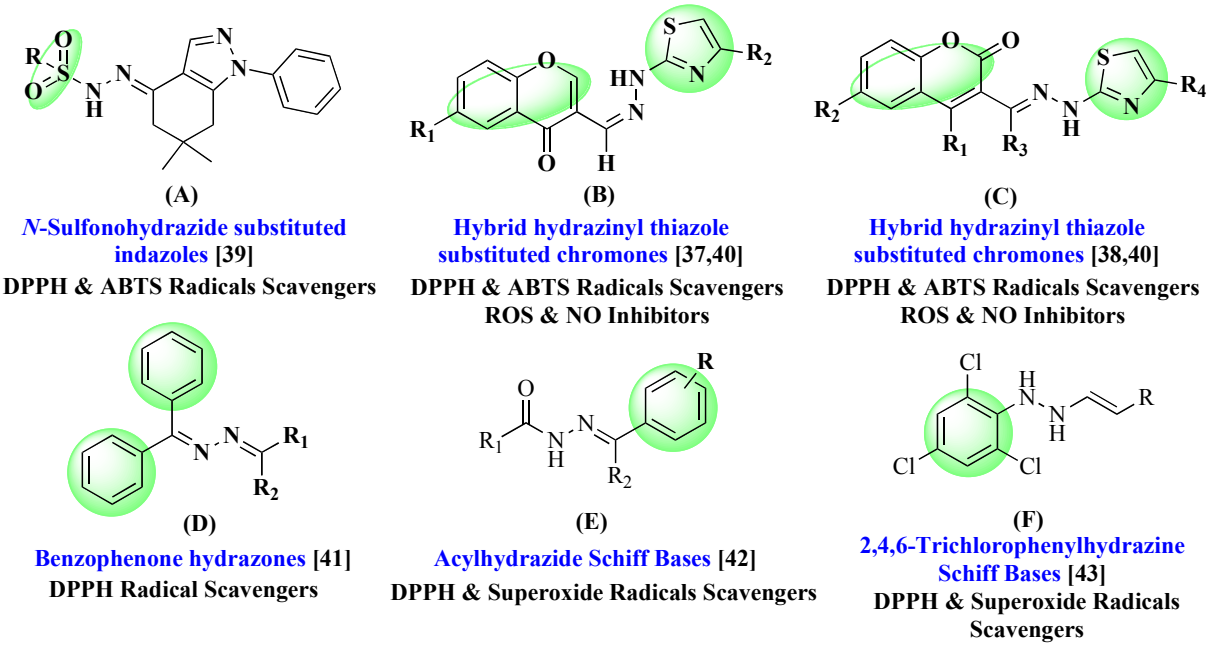


Fig. (6). Previously identified radical scavengers.

worth mentioning that many analogs of both classes (**B** & **C**) were also recognised as ROS and NO (nitric oxide radical) inhibitors [44]. In both cases, methylene hydrazine linker might play an important role in radical scavenging events but it is worth noting that two aromatic systems *i.e.* coumarin/chromone and thiazole on either side of the linker were also involved in the stabilization of free radicals by extensive conjugation (Fig. **6**). While in the case of chalcones and *bis*-chalcones, two aromatic systems on either side of the enone linker, definitely played a part in the stabilization of free radical. Furthermore, different libraries of Schiff bases having diversely substituted aromatic systems linked to them were also found to be excellent DPPH and superoxide anion scavengers [45-47] (Fig. **6**). As shown before, synthetic chalcones and *bis*-chalcones demonstrated DPPH (IC_{50} = 0.58 - 1.72 μM) and ABTS (IC_{50} = 0.49 - 1.48 μM) radical scavenging results better than the previously identified leads due to two aromatic rings attached to enone (linker), which facilitates the stabilization of free radicals by resonance. Past results encouraged us to further evaluate chalcones and *bis*-chalcones for their scavenging or inhibitory potential against ROS and NO radicals in order to obtain a series of potential antioxidants.

CONCLUSION

Synthetic chalcones and *bis*-chalcones **1-18** were evaluated for DPPH and ABTS radical scavenging activities *in vitro*. All synthetic compounds especially compounds bear-

ing -SMe and -OMe groups showed pronounced DPPH and ABTS radical scavenging potential. These compounds with dual potential (α-amylase inhibitors and radical scavengers) have immense strength to serve as leads for antidiabetic activity.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All the relevant data has already been provided. There is no further data to reveal.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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