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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, docking studies and antioxidant activity of some chalcone and aurone derivatives

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Abstract Chalcones and aurones are found to possess high antioxidant activity. They are known to inhibit tyrosinase enzyme involved in synthesis of melanin. A series of substituted chalcones and aurones have been synthesized and tested for their antioxidant activity. Postulated structures of the newly synthesized compounds are in agreement with their IR, ^{1}H NMR and MS. The docking study of this series of compounds was performed on crystal structure of tyrosinase from *Bacillus megaterium* using VlifeMDS 3.0 software. Antioxidant activity data obtained from four methods, i.e., DPPH free radical scavenging assay, iron chelating assay, reducing power assay and hydrogen peroxide scavenging assay, indicate that the activity increased with dimethylamino group on position 4/4′ of ring B as evident from the significant activities of **SB7** and **SB8** in case of chalcones and aurones, respectively. The poor activities of **SB4** and **SB5** in DPPH scavenging ability and reducing power assays could be because of presence of chloro group on B-ring. Furthermore, the activity is facilitated with the presence of hydroxyl group on A-ring (preferably on position 5/5′) in both chalcones and aurones.

Keywords Chalcones · Aurones · Antioxidant · Docking · Tyrosinase

Introduction

The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Free radicals are responsible for causing a large number of diseases including cancer (Kinnula and Crapo, 2004), cardiovascular diseases (Singh and Jialal, 2006), neural disorders (Sas *et al.*, 2007), Alzheimer's disease (Smith *et al.*, 2000), mild cognitive impairment (Guidi *et al.*, 2006), Parkinson's disease (Bolton *et al.*, 2000), alcohol-induced liver disease (Arteel, 2003), ulcerative colitis (Ramakrishna *et al.*, 1997), aging (Hyun *et al.*, 2006), and atherosclerosis (Upston *et al.*, 2003). Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery.

Therefore, antioxidants are intensively used, particularly as treatment for stroke and neuro-degenerative diseases. Antioxidants anti aging benefits are found to be derived from the antioxidants' ability to heal the cell and prevent the free radicals from reproducing. Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease. In addition to these uses of natural antioxidants in medicine, these compounds have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline. As a result, compounds possessing antioxidant activity are becoming increasingly important in disease prevention and therapy (Hamid *et al.*, 2010).

The chalcone and aurone derivatives have displayed a broad spectrum of pharmacological activities including antioxidant activities. Changes in their structure have offered a high degree of diversity that has proven useful for the development of new antioxidants having improved potency and lesser toxicity. Also the nucleus has been

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found to be used as antioxidant in a wide range of formulations targeting for the antiaging activity.

Chalcones, 1,3-diaryl-2-propen-1-ones (Fig. 1) precursors of open chain flavonoids and isoflavonoids present in edible plants, have attracted increasing attention due to numerous potential pharmacological applications. They have displayed a broad spectrum of pharmacological activities, among which antimalarial, anticancer (Lawrence *et al.*, 2006; Cabrera *et al.*, 2007; Boumendjel *et al.*, 2008), antiprotozoal (antileishmanial and antitrypanosomal), anti-inflammatory, antibacterial (Sugamoto *et al.*, 2008; Fvila *et al.*, 2008), antifilarial, antifungal, antimicrobial, larvicidal, anticonvulsant, antioxidant (Stevens *et al.*, 2003; Gacche *et al.*, 2007; Vogel *et al.*, 2008; Jung *et al.*, 2008) activities have been reported. Chalcone derivatives are found to inhibit tyrosinase enzyme involved in melanin production. In chalcones, antioxidant activity is attributable to phenolic–OH group attached to the ring structure (Okawa *et al.*, 2001).

Aurones, 2-benzylidenebenzofuran-3-(2H)-ones (Fig. 1) occur rarely in nature and are less studied subclass of flavonoids. Aurones contribute to the bright yellow color of some flowering plants some such as snapdragon, cosmos and dahlia. They are biosynthesized from chalcones in the presence of enzyme aureusidin synthase (Ono *et al.*, 2006). These heterocyclic compounds are found to possess insect antifeedant activity, anticancer (Lawrence *et al.*, 2003; Vogel *et al.*, 2008), antileishmanial (Huang et al., 2007) and antibacterial properties (Ferreira *et al.*, 2004), inhibitory activity against a variety of enzymes and proteins, however only few study exist targeting their antioxidant activity (Hadj-esfandiari *et al.*, 2007; Venkateswarlu *et al.*, 2004, 2009).

This work is thus aimed to synthesize and evaluate some chalcone and aurone derivatives for their antioxidant activity. The synthesized compounds were also docked on the enzyme tyrosinase. Docking procedures basically aim to identify the correct conformation of ligands in the binding pocket of a protein and to predict the affinity between the ligand and the protein. Docking is one method in which the binding of an inhibitor to a receptor can be explored (Dominguez *et al.*, 2003; Khan, 2012; Okombi *et al.*, 2006; Dubois *et al.*, 2012).

Materials and methods

Physical measurements

Melting point of all the compounds was determined by open capillary melting point apparatus. Thin layer chromatography was performed by using prepared precoated silica gel-G TLC plates. The IR spectra were recorded on FT-IR, Shimadzu-8400S at School of Pharmacy, DAVV, Indore (MP). Mass spectra of compounds were obtained on Bruker microTOF-QII 10348 mass spectrometer using ESI source at IIT, Indore (MP), Micromass Quattro II using ESI source at Sophisticated Analytical Instrumentation Facility (SAIF), CDRI, Lucknow and LC/MS/MS at RGPV, Bhopal. ^{1}H-NMR spectra of compounds were obtained on Bruker Advance II 400 NMR spectrometer at Sophisticated Analytical Instrumentation Facility (SAIF), Panjab University and Bruker DRX-300(300 MHz FT NMR) at Sophisticated Analytical Instrumentation Facility (SAIF), CDRI, Lucknow. For the in vitro tests, a Shimadzu UV–Vis double beam spectrophotometer was used.

Synthesis of compounds

Synthesis of chalcones

To a stirred solution of the appropriate acetophenone (1 equiv) and a substituted benzaldehyde (1 equiv) in 25 ml ethanolic KOH (20 % w/v aqueous solution, 6 ml) was added and the mixture was stirred at room temperature for 24–36 h. The reaction was monitored by performing TLC using n-hexane: ethyl acetate (7:3) as mobile phase. The reaction mixture was cooled to 0 °C (ice-water bath) and acidified with HCl (10 % v/v aqueous solution). In most cases, a yellow precipitate was formed, which was filtered and washed with 10 % aqueous HCl solution. In the cases where an orange oil was formed, the mixture was extracted

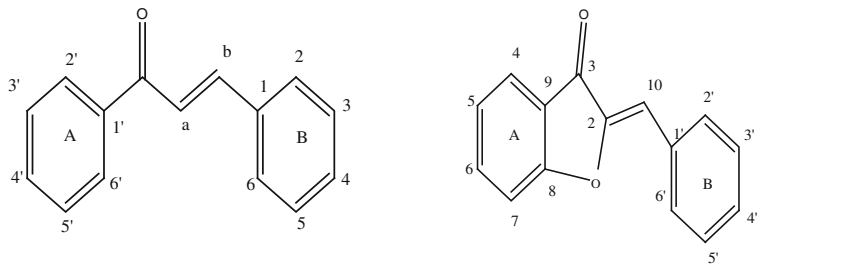


Fig. 1 General structure of chalcone and aurone

1,3-diaryl-2-propen-1-ones (Chalcones) **2-benzylidenebenzofuran-3-(2H)-ones (Aurones)**

with CH_2Cl_2, the extracts were dried (Na_2SO_4) and the solvent was evaporated to give the chalcone (Scheme 1; Table 1) as a solid (Detsi *et al.*, 2009).

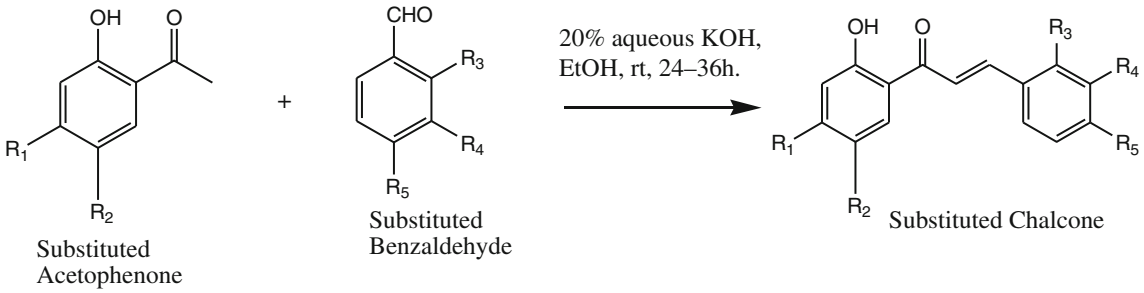
Synthesis of aurones

The substituted chalcone (0.002 mol) was dissolved in dimethyl sulfoxide (DMSO) (10 ml) and after adding mercuric acetate (0.003 mol); the contents were refluxed for 6 h, cooled and poured into ice-cold water. The reaction was monitored by performing TLC using *n*-hexane:ethyl acetate (7:3) as mobile phase (Bhasker and Reddy 2011; Barros *et al.*, 2004). A solid mass separated out which was filtered, washed well with water, dried, and crystallized from ethanol to get aurones (Scheme 2; Table 2).

2′,5′-Dihydroxy-3,4-dimethoxy chalcone ***(SB1)*** Prepared following the general procedure starting from 2,5-dihydroxy-acetophenone (1.52 g, 0.01 mol) and 3,4-dimethoxy benz-aldehyde (1.66 g, 0.01 mol). The yellowish orange product was recrystallized from ethanol. Yield: 42 %; $R_f = 0.64$; m.p. 118–120 °C; IR (KBr) ν (cm^{-1}): 3060.82 (CH–Ar), 1604.66 (CH=CH), 1693.38 (C=O), 3274.9 [O–H (aromatic)], 2835.16 (O–CH_3), 1257.5 [C–O (aromatic)]; ^{1}H-NMR (300 MHz, DMSO) δ: 13.3(s, 1H, –OH group on C-2′), 2.50 (s, 1H, –OH group on C-5′), 7.73 (d, 1H, H_a), 7.82 (d, 1H, H_b), 6.88 (d, 1H, H-5), 6.96 (d, 1H, H-6), 7.01 (d, 1H, H-3′), 7.38 (d, 1H, H-4′), 7.14 (s, 1H, H-2), 7.55 (s, 1H, H-6′), 3.80 (s, 3H, –OCH_3 group on C-3), 3.81 (s, 3H, –OCH_3 group on C-4); MS (LC/MS) *m/z*: 301.13 $(M+H)^+$; Mol. formula: $C_{17}H_{16}O_5$; Mol. Weight: 301.31.

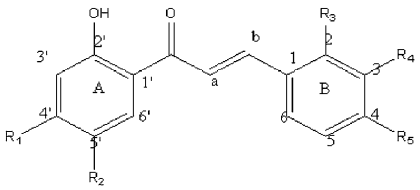
2′,4′-Dihydroxy-4-dimethylamino chalcone ***(SB2)*** Prepared following the general procedure starting from 2, 4-dihydroxy-acetophenone (1.52 g, 0.01 mol) and 4-dimethylamino benzaldehyde (1.49 g, 0.01 mol). The dark orange product was recrystallized from ethanol. Yield: 57 %; $R_f = 0.62$; m.p. 150–152 °C; IR (KBr) ν (cm^{-1}): 3105.18 (CH–Ar), 1593.09 (CH=CH), 1694 (C=O), 3328.91 [O–H (aromatic)], 2781.16 (N–CH_3), 1232.43 [C–O (aromatic)]. ^{1}H-NMR (300 MHz, DMSO) δ: 12.5 (s, 1H, –OH group on C-2′), 9.67 (s, 1H, –OH group on C-4′), 7.70 (d, 1H, H_a), 7.93 (d, 1H, H_b), 6.24 (s, 1H, H-3′), 6.35 (d, 1H, H-5′), 6.38 (d, 2H, H-3, H-5), 6.77 (d, 2H, H-2, H-6), 7.67 (d, 1H, H-6′), 3.04 (s, 6H, $N(CH_3)_2$ group on C-4); MS (LC/MS) *m/z*: 284.15 $[M+H]^+$; Mol. formula: $C_{17}H_{17}O_3$; Mol. Weight: 284.32.

6-Hydroxy-4′-dimethylamino aurone ***(SB3)*** Prepared following the general procedure starting from 2′,

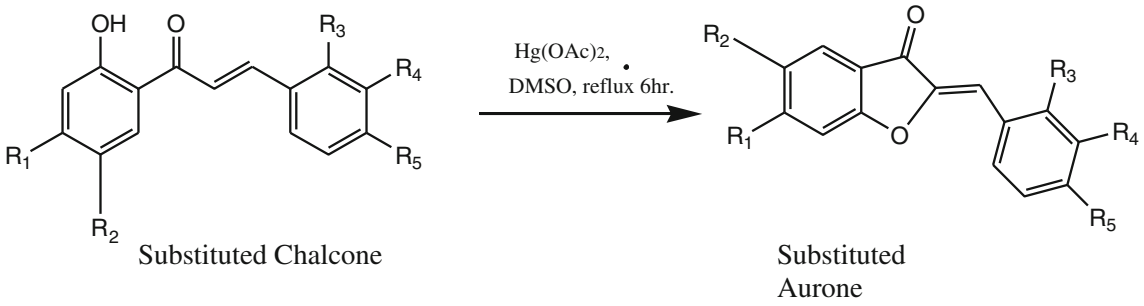


Scheme 1 Synthesis of chalcones

Table 1 Substituted chalcones

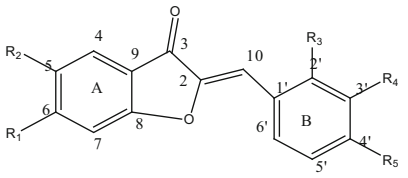


S no.	Compounds	R_1	R_2	R_3	R_4	R_5
SB1	2′,5′-Dihydroxy-3,4-dimethoxy chalcone	H	OH	H	OCH_3	OCH_3
SB2	2′,4′-Dihydroxy-4-dimethylamino chalcone	OH	H	H	H	$N(CH_3)_2$
SB5	2′,4′-Dihydroxy-4-chloro chalcone	OH	H	H	H	Cl
SB7	2′,5′-Dihydroxy-4-dimethylamino chalcone	H	OH	H	H	$N(CH_3)_2$
SB10	2′,4′-Dihydroxy-3,4-dimethoxy chalcone	OH	H	H	OCH_3	OCH_3



Scheme 2 Synthesis of aurones

Table 2 Substituted aurones



S no.	Compounds	R_1	R_2	R_3	R_4	R_5
SB3	6-Hydroxy-4′-dimethylamino aurone	OH	H	H	H	$N(CH_3)_2$
SB4	6-Hydroxy-4′-chloro aurone	OH	H	H	H	Cl
SB6	5-Hydroxy-3′,4′-dimethoxy aurone	H	OH	H	OCH_3	OCH_3
SB8	5-Hydroxy-4′-dimethylamino aurone	H	OH	H	H	$N(CH_3)_2$
SB9	6-Hydroxy-3′,4′-dimethoxy aurone	OH	H	H	OCH_3	OCH_3

4′-dihydroxy-4-dimethylamino chalcone (566 mg, 0.002 mol). The orange brown product was recrystallized from ethanol. Yield: 43 %; $R_f = 0.58$; m.p. 98–100 °C; IR (KBr) ν (cm^{-1}): 3060.82 (CH–Ar), 1581.52 (CH=CH), 1693.38 (C=O), 3487.06 [O–H (aromatic)], 2796.59 (N–CH_3), 1245.93 [C–O (aromatic)]; ^{1}H-NMR (300 MHz, DMSO) δ: 10.59 (s, 1H, –OH group on C-6), 7.76 (s, 1H, H-10), 6.24 (s, 1H, H-7), 6.36 (d, 1H, H-5), 6.39 (d, 2H, H-3′, H-5′), 6.80 (d, 2H, H-2′, H-6′), 7.70 (d, 1H, H-4), 3.30 (s, 6H, $N(CH_3)_2$ group on C-4′); MS (LC/MS) *m/z*: 282.13 $[M+H]^+$; Mol. formula: $C_{17}H_{15}O_3N$; Mol. Weight: 282.31.

*6-Hydroxy-4′-chloro aurone (**SB4**)* Prepared following the general procedure starting from 2′,4′-dihydroxy-4-chloro chalcone (549 mg, 0.002 mol). The yellow product was recrystallized from ethanol. Yield: 54 %; $R_f = 0.74$; m.p. 120–125 °C; IR (KBr) ν (cm^{-1}): 3049.25 (CH–Ar), 1593.09 (CH=CH), 1674.1 (C=O), 3562.28 [O–H (aromatic)], 769.54 (C–Cl), 1261.36 [C–O (aromatic)]; ^{1}H-NMR (300 MHz, DMSO) δ: 7.79 (s, 1H, –OH group on C-6), 7.05 (s, 1H, H-10), 6.30 (s, 1H, H-7), 5.59 (d, 1H, H-5), 6.51 (d, 2H, H-3′, H-5′), 7.64 (d, 2H, H-2′, H-6′), 7.74 (d, 1H, H-4); MS (LC/MS) *m/z*: 275.06 $[M+H+2]^+$; Mol. formula: $C_{15}H_9O_3Cl$; Mol. Weight: 275.68.

*2′,4′-Dihydroxy-4-chloro chalcone (**SB5**)* Prepared following the general procedure starting from 2,4-dihydroxy-acetophenone (1.52 g, 0.01 mol) and 4-chloro benzaldehyde (1.4 g, 0.01 mol). The yellow product was recrystallized from ethanol. Yield: 76 %; $R_f = 0.78$; m.p. 155–160 °C; IR (KBr) ν (cm^{-1}): 3089.75 (CH–Ar), 1585.38 (CH=CH), 1691.46 (C=O), 3575.78 [O–H (aromatic)], 763.76 (C–Cl), 1215.07 [C–O (aromatic)]; ^{1}H-NMR (300 MHz, DMSO) δ: 13.3 (s, 1H, –OH group on C-2′), 2.50 (s, 1H, –OH group on C-4′), 7.74 (d, 1H, H_a), 8.18 (d, 1H, H_b), 6.80 (s, 1H, H-3′), 6.95 (d, 1H, H-5′), 7.44 (d, 2H, H-3, H-5), 7.64 (d, 2H, H-2, H-6), 7.59 (d, 1H, H-6′); MS (ESI) *m/z*: 277.05 $[M+H+2]^+$; Mol. formula: $C_{15}H_{11}O_3Cl$; Mol. Weight: 277.7.

*5-Hydroxy-3′,4′-dimethoxy aurone (**SB6**)* Prepared following the general procedure starting from 2′,5′-dihydroxy-3′,4′-dimethoxy chalcone (600 mg, 0.002 mol). The dark orange product was recrystallized from ethanol. Yield: 38 %; $R_f = 0.61$; m.p. 110–112 °C; IR (KBr) ν (cm^{-1}): 3105.18 (CH–Ar), 1544.88 (CH=CH), 1685.67 (C=O),

3458.13 [O–H (aromatic)], 2829.38 (s, O–CH_3), 1249.79 [C–O (aromatic)]; ^{1}H-NMR (300 MHz, DMSO) δ: 9.70 (s, 1H, –OH group on C-5), 7.19 (s, 1H, H-10), 7.81 (d, 1H, H-7), 7.40 (d, 1H, H-6), 7.47 (d, 1H, H-5′), 7.56 (d, 1H, H-6′), 7.52 (s, 1H, H-4), 7.75 (s, 1H, H-2′), 3.44 (s, 3H, –OCH_3 group on C-3′), 3.48 (s, 3H, –OCH_3 group on C-4′); MS (ESI) *m/z*: 300.9 $[M+H+H]^+$; Mol. formula: $C_{17}H_{14}O_5$; Mol. Weight: 300.29.

2′,5′-Dihydroxy-4-dimethylamino chalcone ***(SB7)*** Prepared following the general procedure starting from 2, 5-dihydroxy-acetophenone (1.52 g, 0.01 mol) and 4-dimethylamino benzaldehyde (1.49 g, 0.01 mol). The brown product was recrystallized from ethanol. Yield: 62 %; $R_f = 0.67$; m.p. 98–100 °C; IR (KBr) ν (cm^{-1}): 3035.75 (CH–Ar), 1581.52 (CH=CH), 1706.88 (C=O), 3433.06 (O–H (aromatic)), 2796.59 (N–CH_3), 1218.93 [C–O (aromatic)]; ^{1}H-NMR (300 MHz, DMSO) δ: 12.6 (s, 1H, –OH group on C-2′), 9.67 (s, 1H, –OH group on C-5′), 7.65 (d, 1H, H_a), 8.06 (d, 1H, H_b), 6.24 (d, 1H, H-3′), 6.34 (d, 1H, H-4′), 6.72 (d, 2H, H-3, H-5), 6.75 (d, 2H, H-2, H-6), 6.36 (s, 1H, H-6′), 3.07 (s, 6H, N(CH_3)$_2$ group on C-4); MS (ESI) *m/z*: 283.9 $[M]^+$; Mol. formula: $C_{17}H_{17}O_3N$; Mol. Weight: 283.32.

5-Hydroxy-4′-dimethylamino aurone ***(SB8)*** Prepared following the general procedure starting from 2′,5′-dihydroxy-4-dimethylamino chalcone (566 mg, 0.002 mol). The brown product was recrystallized from ethanol. Yield: 49 %; $R_f = 0.64$; m.p. 110–112 °C; IR (KBr) ν (cm^{-1}): 3153.4 (CH–Ar), 1589.23 (CH=CH), 1637.45 (C=O), 3336.62 [O–H (aromatic)], 2806.23 (N–CH_3), 1220.86 [C–O (aromatic)]; ^{1}H-NMR (300 MHz, DMSO) δ: 12.45 (s, 1H, –OH group on C-5), 7.54 (s, 1H, H-10), 7.30 (1H, H-6), 6.83 (d, 1H, H-7), 7.14 (d, 2H, H-3′, H-5′), 7.46 (d, 2H, H-2′, H-6′), 7.20 (s, 1H, H-4), 3.06 (s, 6H, N(CH_3)$_2$ group on C-4′); MS (ESI) *m/z*: 281 $[M]^+$; Mol. formula: $C_{17}H_{15}O_3N$; Mol. Weight: 281.31.

6-Hydroxy-3′,4′-dimethoxy aurone ***(SB9)*** Prepared following the general procedure starting from 2′,6′-dihydroxy-3′,4′-dimethoxy chalcone (600 mg, 0.002 mol). The yellowish orange product was recrystallized from ethanol. Yield: 42 %; $R_f = 0.49$; m.p. 118–120 °C; IR (KBr) ν (cm^{-1}):3028.03 (CH–Ar), 1554.52 (CH=CH), 1695.31 (C=O), 3595.07 [O–H (aromatic)], 2866.02 (O–CH_3), 1288.36 [C–O (aromatic)]; ^{1}H-NMR (300 MHz, DMSO) δ: 13.53 (s, 1H, -OH group on C-6), 6.97 (s, 1H, H-10), 6.91 (s, 1H, H-7), 6.29 (d, 1H, H-5), 7.08 (d, 1H, H-5′), 7.44 (d, 1H, H-6′), 7.73 (d, 1H, H-4), 7.78 (s, 1H, H-2′), 3.92 (s, 3H, –OCH_3 group on C-3′), 3.87 (s, 3H, –OCH_3 group on C-4′); MS (ESI) *m/z*: 298.8 $[M]^+$; Mol. formula: $C_{17}H_{14}O_5$; Mol. Weight: 298.29.

2′,4′-Dihydroxy-3,4-dimethoxy chalcone ***(SB10)*** Prepared following the general procedure starting from 2, 4-dihydroxy-acetophenone (1.52 g, 0.01 mol) and 3,4-dimethoxy benzaldehyde (1.66 g, 0.01 mol). The yellowish orange product was recrystallized from ethanol. Yield: 43 %; $R_f = 0.52$; m.p. 120–125 °C; IR (KBr) ν (cm^{-1}): 3078.18 (CH–Ar), 1485.09 (CH=CH), 1693.38 (C=O), 3558.42 [O–H (aromatic)], 2846.74 (O–CH_3), 1213.14 [C–O(aromatic)]; ^{1}H-NMR (300 MHz, DMSO) δ: 13.5 (s, 1H, –OH group on C-2′), 10.45 (s, 1H, –OH group on C-4′), 7.31 (d, 1H, H_a), 7.46 (d, 1H, H_b), 6.28 (d, 1H, H-5), 7.29 (d, 1H, H-6), 6.92 (s, 1H, H-3′), 6.39 (d, 1H, H-5′), 7.75 (s, 1H, H-2), 6.42 (d, 1H, H-6′), 3.82 (s, 3H, –OCH_3 group on C-3), 3.84 (s, 3H, –OCH_3 group on C-4); MS (ESI) *m/z*: 301.21 $[M+H]^+$; Mol. formula: $C_{17}H_{16}O_5$; Mol. Weight: 301.31.

Docking study on tyrosinase using VlifeMDS 3.0

To explore the interactions of the synthesized compounds, we carried out binding simulations by means of the Bio-Predicta module of Vlife MDS 3.0 suite (Vlife MDS, 2006). The docking study (Kang *et al.*, 2012) was performed on crystal structure of tyrosinase from *Bacillus megaterium* (PDB code: 3NM8). The computational work was performed on a HP Compaq PC running on Intel core2duo processor. The molecular structures of the compounds in the data set were sketched using VLife MDS (Molecular Design Suite) 3.0 software supplied by VLife Sciences Technologies Pvt. Ltd., Pune, India. Energy minimization was performed using the MMFF94 (Halgren, 1996) force field and Gasteiger–Marsili (Gasteiger and Marsili, 1980) charges followed by AM-1 (Austin Model-1) Hamiltonian method available in MOPAC module with the convergence criterion 0.001 kcal/mol Å.

The 3D structure of the tyrosinase was retrieved from PDB by giving the PDB ID (PDB entry 3NM8; www.rcsb.org) in the database. The water molecules were removed and hydrogen atoms were added. The docking simulations were done and potential hydrogen bonding, pi-stacking, VDW, and hydrophobic interactions between the protein and the synthesized compounds were recorded. The water molecules were removed and hydrogens were added. The energy of the protein was minimized using MMFF.

The ligands were prepared using Prepare Ligands and subsequently docked using Grid Docking. All the docked ligands were scored using the Dock Score function. The best pose was identified and used for subsequent analyses.

In vitro assays

Each in vitro experiment was performed at least in triplicate. Ascorbic acid was used as the standard in all the four methods.

DPPH free radical scavenging activity

0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of solution of compound in methanol at different concentrations (50, 100, 150, 200, 250, 300 μg/ml). Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared without adding compound. Ascorbic acid at various concentrations (50–300 μg/ml) was used as standard. Lower absorbance value of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\% \text{ Antiradical activity} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

The antioxidant activity of compounds (Table 3) were expressed as IC_{50} and compared with standard (Nikhat and Satyanarayana 2009).

Iron chelating activity

Iron chelating activity (Table 3) is a measure of antioxidant activity. Different concentrations of compound (50–300 μg/ml) and ascorbic acid solution (50–300 μg/ml) each as 2 ml in methanol were incubated with methanolic *o*-phenanthroline solution (1 ml, 0.05 % w/v) and ferric chloride solution (2 ml, 200 μM) at ambient temperature for 10 min. After incubation, the absorbance of solutions was measured at 510 nm (Benzie and Strain, 1996).

Hydrogen peroxide scavenging assay

The solution of hydrogen peroxide (100 mM) was prepared by the addition of various concentrations of compound (50–300 μg/ml) to hydrogen peroxide solution (2 ml) in phosphate buffer saline of pH 7.4. Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. For control sample, absorbance of hydrogen peroxide solution was taken at 230 nm (Patel and Patel, 2010). The percentage inhibition activity (Table 3) was calculated from the formula $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of test/standard taken as ascorbic acid (50–300 μg/ml).

Reducing power method

2 ml of each sample and standard solutions were spiked with 2.5 ml of 1 % potassium ferricyanide solution. This mixture was kept at 50 °C in water bath for 20 min. After cooling, 2.5 ml of 10 % trichloroacetic acid was added, (when precipitate formed, centrifuged at 3,000 rpm for 10 min) 2.5 ml of supernatant was mixed with 2.5 ml of distilled water and 1 ml of 0.1 % ferric chloride and kept for 10 min. Control was prepared in similar manner excluding samples (Collins, 2005). The absorbance of resulting solution was measured at 700 nm (Table 3).

Results and discussion

Chemistry

Substituted chalcones (**SB1**, **SB2**, **SB5**, **SB7**, **SB10**) have been synthesized via the Claisen–Schmidt condensation reaction between appropriately substituted acetophenones and benzaldehydes in basic conditions (20 % aqueous KOH) (Scheme 1; Table 1). The synthesis of the desired aurones (**SB3**, **SB4**, **SB6**, **SB8**, **SB9**) was accomplished via the oxidative cyclization methodology using mercury(II)

Table 3 Antioxidant activity of substituted chalcones and aurones

Compound no.	DPPH scavenging ability IC_{50} μg/ml ± SD	H_2O_2 scavenging ability IC_{50} μg/ml ± SD	Iron chelating activity assay IC_{50} μg/ml ± SD	Reducing power IC_{50} μg/ml ± SD
SB1	132.22 ± 0.7	89.59 ± 1.8	159.22 ± 1.02	111.79 ± 0.69
SB2	65.8 ± 0.7	135.88 ± 0.53	140.16 ± 1.08	53.6 ± 1.1
SB3	109.92 ± 1.02	193.91 ± 0.36	179.33 ± 0.31	70.1 ± 2.2
SB4	266.12 ± 0.49	178.13 ± 0.36	179.18 ± 0.74	188.31 ± 0.31
SB5	264.95 ± 1.6	129.02 ± 0.85	159.97 ± 0.29	155.87 ± 0.68
SB6	222.19 ± 0.7	206.57 ± 0.19	185.05 ± 1.2	151.43 ± 0.25
SB7	24.32 ± 0.87	153.85 ± 2.1	90.81 ± 0.86	47.79 ± 1.3
SB8	243 ± 0.13	174.07 ± 1.9	149.98 ± 0.51	64.16 ± 1.2
SB9	173.29 ± 0.94	248.16 ± 0.76	213.83 ± 0.68	168.23 ± 0.83
SB10	35.2 ± 1.5	70.1 ± 0.92	166.73 ± 2.5	134.45 ± 0.34
Ascorbic acid	98.77 ± 0.53	445.92 ± 1.4	126.12 ± 0.5	53.24 ± 0.72

acetate in dimethyl sulfoxide (Scheme 2; Table 2) The compounds were purified by recrystallization. In all cases, the compounds were obtained in moderate to satisfactory yields (38–76 %). Postulated structures of the newly synthesized compounds are in agreement with their IR, ^{1}H NMR and MS.

Molecular docking

Docking was performed on crystal structure of tyrosinase from *B. megaterium* (PDB code: 3NM8) using VLife MDS 3.0 software. Docking studies showed that Ile39A, Gly143A, Ile139A, Lys47A, Lys47B, Ala44B, Gly43B, and Gln142A present in tyrosinase are highly conserved and may play a major role in substrate binding or catalysis. Standard nordihydroguaiaretic acid (NDGA) is also found to bind to these amino acids (Fig. 2). Some other conserved residues surrounding the binding site are His49B and Glu141B.

2′,4′-Dihydroxy-4-chloro chalcone, **SB5** (Table 4; Fig. 3) has shown dock score (−40.19 kcal/mole) comparable to the standard (−41.88 kcal/mol), while 5-hydroxy-4′-dimethylamino aurone, **SB8** (Fig. 4) displayed better dock score (−91.39 kcal/mol) compared to NDGA (−41.88 kcal/mol).

Antioxidant activity

All synthesized compounds were evaluated for invitro antioxidant activity (Table 3) using four different antioxidant assays. The radical scavenging ability of the compounds was tested against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable free radical and hydrogen peroxide. Also the measurement of the reducing ability, the Fe^{3+}–Fe^{2+} transformation was investigated along with iron chelating ability.

Table 4 Dock score of substituted chalcone and aurone derivatives

Compound no.	Compounds	Dock score (kcal/mol)
SB1	2′,5′-Dihydroxy-3,4-dimethoxy chalcone	−5.35
SB2	2′,4′-Dihydroxy-4-dimethylamino chalcone	−25.62
SB3	6-Hydroxy-4′-dimethylamino aurone	−42.23
SB4	6-Hydroxy-4′-chloro aurone	−40.89
SB5	2′,4′-Dihydroxy-4-chloro chalcone	−40.19
SB6	5-Hydroxy-3′,4′-dimethoxy aurone	−34.43
SB7	2′,5′-Dihydroxy-4-dimethylamino chalcone	−31.95
SB8	5-Hydroxy-4′-dimethylamino aurone	−91.39
SB9	6-Hydroxy-3′,4′-dimethoxy aurone	−31.82
SB10	2′,4′-Dihydroxy-3,4-dimethoxy chalcone	−16.91
Standard	NDGA (Nordihydroguaiaretic acid)	−41.88

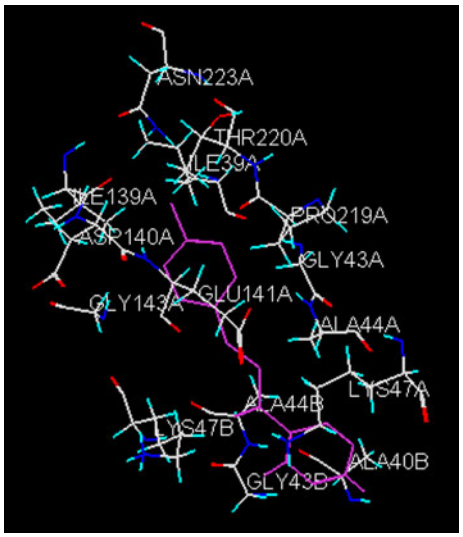


Fig. 3 Docking view of 2′,4′-dihydroxy-4-chloro chalcone (**SB5**)

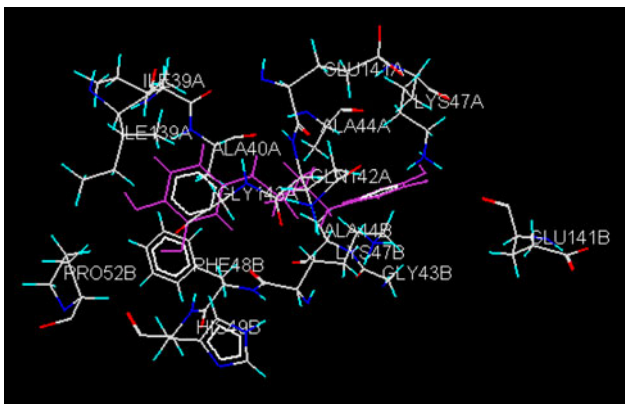


Fig. 2 Docking view of NDGA

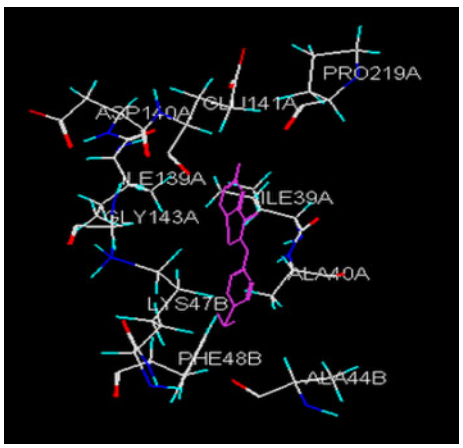


Fig. 4 Docking view of 5-hydroxy-4′-dimethylamino aurone (**SB8**)

DPPH free radical scavenging activity

The interaction of the synthesized compounds with the stable free radical DPPH indicates their radical scavenging ability in an iron-free system. The interaction of the tested chalcones and aurones with DPPH was found to be concentration dependent. Among the tested compounds, the best DPPH radical scavenging activity (Table 3) was presented by **SB7** containing *p*-dimethylamino group (IC_{50} 24.32 ± 0.87 μg/ml). Also, **SB10** (IC_{50} 35.2 ± 1.5 μg/ml) and **SB2** (IC_{50} 65.8 ± 0.7 μg/ml) exhibited higher activity than the reference compound ascorbic acid (IC_{50} 98.77 ± 0.53 μg/ml). **SB1** (IC_{50} 132.22 ± 0.7 μg/ml), and **SB3** (IC_{50} 109.92 ± 1.02 μg/ml) exhibited satisfactory activity compared to reference compound. The activity seems to increase with the presence of electron-donating substituents on B-ring. Electron-withdrawing substituents do not favor activity.

Iron chelating activity

Iron is essential for life because it is required for oxygen transport, respiration, and activity of many enzymes. However, iron is an extremely reactive metal and catalyzes oxidative changes in lipids, proteins, and other cellular components (Satheeshkumar *et al.*, 2011). Iron binding capacity of the synthesized compounds and the reference compound ascorbic acid were examined (Table 3). Maximum chelating of metal ions was found to be shown by **SB7** (IC_{50} 90.81 ± 0.86 μg/ml) which was higher than the reference compound. Compounds **SB2** (IC_{50} 140.16 ± 1.08 μg/ml) and **SB8** (IC_{50} 149.98 ± 0.51 μg/ml) exhibited satisfactory activity compared to reference compound. Iron chelating activity seems to depend on the position of the hydroxyl group on ring A (compare **SB7** > **SB2**, **SB1** > **SB10**, and **SB8** > **SB3**). On the other hand, the activity seems to increase with the presence of electron-donating substituents on ring B. There is a correlation between the activity of chalcones and the corresponding aurones in this assay: a highly active chalcone gives a highly active aurone (e.g., **SB7** cyclized to **SB8** and these compounds are highly active). The IC_{50} value of the reference compound ascorbic acid was recorded as 126.12 ± 0.5 μg/ml.

Hydrogen peroxide scavenging assay

Scavenging of hydrogen peroxide by synthesized compounds may be attributed to the phenolics, which can donate electrons to hydrogen peroxide thereby neutralizing it to water. The ability of the compounds to effectively scavenge hydrogen peroxide was determined according to the method of Ruch (Nabavi *et al.*, 2008). The compounds were capable of scavenging hydrogen peroxide in a concentration-dependent manner. The scavenging ability of all the compounds was found to be better compared to the reference compound ascorbic acid (IC_{50} 445.92 ± 1.4 μg/ml). Among the tested compounds, the best scavenging activity was presented by **SB10** (IC_{50} 70.1 ± 0.92 μg/ml) and **SB1** (IC_{50} 89.59 ± 1.8 μg/ml). The presence of methoxy group on 3, 4 position of B-ring increases the hydrogen peroxide scavenging activity (Table 3) compared to electron-withdrawing substituents at para position.

Reducing power method

Fe(III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action. In the reducing power assay, the presence of antioxidants in the samples would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex was then monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reducing ability (Nabavi *et al.*, 2008). It was found that the reducing powers of all the synthesized compounds also increased with the increase in their concentrations. The best reducing power was presented by **SB7** containing *p*-dimethylamino group (IC_{50} 47.79 ± 1.3 μg/ml). **SB2** (IC_{50} 53.60 ± 1.1 μg/ml), **SB8** (IC_{50} 64.16 ± 1.2 μg/ml) and **SB3** (IC_{50} 70.10 ± 2.2 μg/ml) exhibited satisfactory activity compared to reference compound (Table 3). The IC_{50} value of the reference compound was recorded as 53.24 ± 0.72 μg/ml. Reducing power seems to depend on the position of the hydroxyl group on ring A (compare **SB7** > **SB2**, **SB1** > **SB10**, and **SB8** > **SB3**). On B-ring, electron-donating substituents seem to increase the activity. There is a correlation between the activity of chalcones and the corresponding aurones in this assay: a highly active chalcone gives a highly active aurone (e.g., **SB7** cyclized to **SB8** and these compounds are highly active).

Conclusion

Among the results obtained from the four methods for the evaluation of antioxidant activity of chalcones (**SB1**, **SB2**, **SB5**, **SB7**, and **SB10**), the compound **SB7** (2′,5′-dihydroxy-4-dimethylamino chalcone) with *p*-dimethylamino group on B-ring and 2′,5′-dihydroxy group on A-ring of chalcone has shown the most promising antioxidant activity in DPPH free radical scavenging assay, iron chelating assay and

reducing power assay. Compound **SB2** (2′,4′-dihydroxy-4-dimethylamino chalcone) and **SB8** (5-hydroxy-4′-dimethylamino aurone) have also shown good activities following **SB7** in iron chelating assay and reducing power assay.

Among the aurones (**SB3**, **SB4**, **SB6**, **SB8**, and **SB9**), **SB8** has shown best activity in iron chelating assay, hydrogen peroxide scavenging assay and reducing power assay. **SB8** has also shown best dock score in case of docking studies. On the other hand, **SB4** (6-hydroxy-4′chloro aurone) is found to be least active in two of the above methods, namely DPPH free radical scavenging assay and reducing power assay.

Antioxidant activity increased with dimethylamino group on position 4/4′ of ring B as evident from the significant activities of **SB7** and **SB8** in case of chalcones and aurones, respectively. The poor activities of **SB4** and **SB5** in DPPH scavenging ability and reducing power assays could be because of presence of chloro group on B-ring. Also, the activity is facilitated with the presence of hydroxyl group on A-ring (preferably on position 5/5′) in both chalcones and aurones.

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