Journal of Saudi Chemical Society (2014) **18**, 121–127

King Saud University

Journal of Saudi Chemical Society

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Synthesis and reducing power assay of methyl semicarbazone derivatives

Manmohan Singhal [a,*], **Arindam Paul** [b], **Hemendra P. Singh** [c]

[a] *School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India*
[b] *G.D. Memorial College of Pharmacy, Jodhpur, Rajasthan, India*
[c] *B.N. College of Pharmacy, Udaipur, Rajasthan, India*

Received 1 May 2011; accepted 4 June 2011
Available online 15 June 2011

KEYWORDS

Chalcones;
Anti-oxidant;
Semicarbazones;
Reducing power

Abstract In the present study we have designed a new pharmacophore 'Chalconesemicarbazone' by pharmacophore hybridization approach of drug design. A series of novel chalconesemicarbazones was synthesized and evaluated for their antioxidant activity by reducing power assay. Most of the compounds were found to be potent antioxidants. Free radicals play an important role in various pathological and xenotoxic effects so antioxidant may have protective role in these pathological conditions. Based on the results of reducing power assay 1-[1-(2,4-dihydroxyphenyl)-3-(2-hydroxyphenyl)allylidene]-4-(4-methylphenyl)semicarbazide (compound **18**) and 1-[1-(2,5-dihydroxyphenyl)-3-(6-hydroxyphenyl)allylidene]-4-(4-methylphenyl)semicarbazide (compound **21**) were the most active lead compounds. It was found that methoxy and hydroxyl substituted chalconesemicarbazones exhibited potent reducing power and unsubstituted compound showed less reducing potential.

1. Introduction

Free radical is an atom or molecule that bears an unpaired electron and is extremely reactive, capable of engaging in rapid change reaction that destabilize other molecules and generate many more free radicals. In plants and animals these free radicals are deactivated by antioxidants. These antioxidants act as an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. The body is constantly exposed to the negative and sometimes lethal effects of oxidants during normal physiological processes. The harmful free radicals such as hydroxyl, peroxyl and the superoxide anion are constantly being produced as a result of metabolic reactions in living systems. On a daily basis, up to 5% of inhaled oxygen may be converted to reactive oxygen species (ROS). These ROS have the ability to bind to cellular structures, and have been implicated in a number of pathological processes such as aging, inflammation, re-oxygenation of ischemic tissues, atherosclerosis, cancer and even Parkinson's disease in men (Setiadi et al., 2003). Two

* Corresponding author. Tel.: +91 9829153193.
E-mail address: manu.research2@gmail.com (M. Singhal).

Peer review under responsibility of King Saud University.
doi:10.1016/j.jscs.2011.06.004

Production and hosting by Elsevier

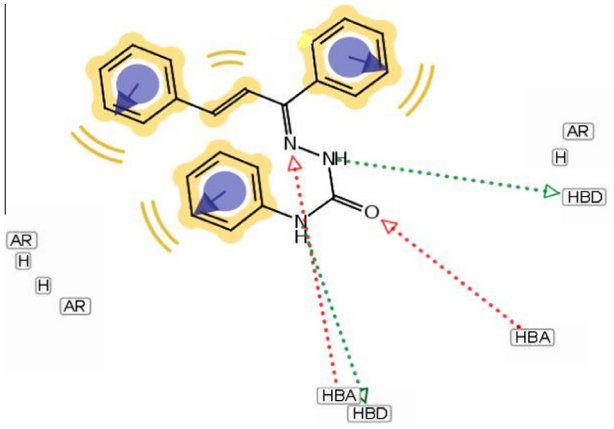

Figure 1 Pharmacophore of the designed chalconesemicarbaz-one.

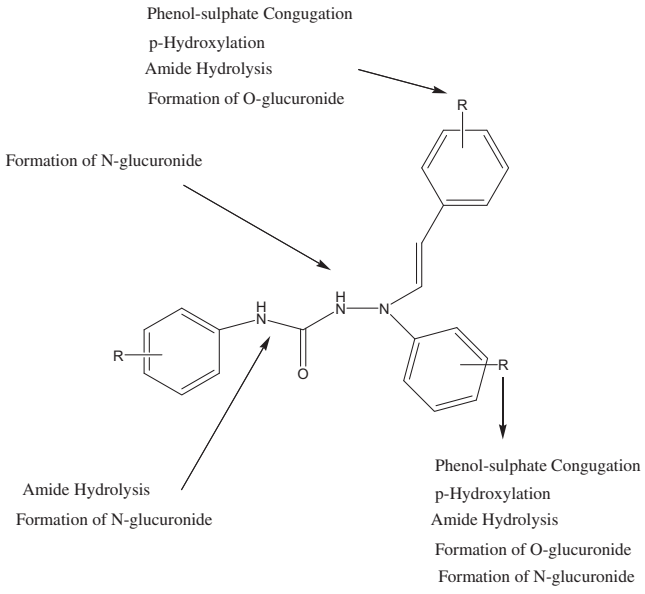

Figure 2 In Silico metabolism of the chalconesemicarbazone.

processes, which produce free radicals in vivo, have been identified and named the Fenton reaction and the Haber–Weiss reaction.

Antioxidants play an important role in animal health. Conventional antioxidants have been shown to improve animal performance during conditions characterized by increased tissue oxidant levels such as stress, injury and infections (Nickels, 2003). The semicarbazone is an electron withdrawing group and exhibited antioxidant activity. Favorable substitution may increase their free radical scavenging effect (Dutta et al., 2005).

In the present study we have used pharmacophore hybridization technique of drug design and designed a pharmacophore model 'chalconesemicarbazone', which is having hydrogen acceptor site, hydrogen donor site, lipophilic site etc. (Fig. 1), which may help in binding with receptors and plays an important role in pharmacological activities. On these observations, we have designed a synthetic scheme to synthesize this pharmacophore, and also synthesize some lead compounds. We have also done the pharmacological screening as antioxidant activity by reducing power assay. No exact mechanism study were done on molecular level but further studies were in process in our lab for searching the exact mechanism of action of these compounds, which may support the showing activities of the synthesized compounds.

In-Silico metabolism prediction of the synthesized compounds is given in Fig. 2. The major pathway of metabolism was found to be p-hydroxylation and amide hydrolysis however in some compounds glucuronide and sulfate conjugation may also occur.

2. Materials and methods

2.1. Chemistry

Chalconesemicarbazones were synthesized according to synthetic scheme as shown in Fig. 3. Melting points were measured in open capillary tubes on a Buchi 530 melting point apparatus and were uncorrected. Infrared (IR) and proton nuclear magnetic resonance (1H NMR) spectra were recorded for the compounds on Jasco IR Report 100 (KBr) and Brucker Advance (300 MHz) instruments, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. All exchangeable protons were confirmed by addition of D_2O. Mass spectra were measured with a Shimadzu GC–MS–QP5000 spectrophotometer. Only molecular ions (M+) and base peaks are given. Elemental analysis (C, H and N) were undertaken with a Perkin-Elmer model 240C analyzer, and all analyses were consistent with theoretical values (within 0.4%) unless indicated. The homogeneity of the compounds was monitored by ascending thin-layer chromatography (TLC) on silica gel G (Merck) coated aluminum plates, visualized by iodine vapor.

2.1.1. Synthesis of substituted chalcone derivatives

Substituted benzaldehydes (0.012 mol) were added to a mixture of substituted acetophenones (0.01 mol) in 25 ml of ethanol in a 200 ml beaker. The content of the beaker was mixed well and to that 10 ml of 10% potassium hydroxide solution was added and stirred vigorously at 25 °C until the mixture was so thick that stirring was no longer effective (3–4 h).

After the completion of the stirring, the reaction mixture was kept in a refrigerator overnight. The reaction mixture was then diluted with ice-cold water (50 ml), acidified with 10% aqueous hydrochloric acid to precipitate the chalcones. The product was filtered with suction on a Buchner funnel, washed with cold water until the washings were neutral to litmus and then washed with 10 ml of ice-cold rectified spirit. The dried product was recrystallized from chloroform. The physicochemical properties of the synthesized chalcone derivatives are given in Table 1.

*2.1.2. Synthesis of methyl phenyl urea (**2**)*

Substituted aniline (0.1 mol) was dissolved in 20 ml of glacial acetic acid and 10 ml of water. To this, 0.1 mol of sodium cyanate (6.5 g) in 80 ml of warm water was added with continuous stirring. The reaction mixture was allowed to stand for 30 min and then cooled in ice. The crude solid, thus obtained was filtered, dried and recrystallized with boiling water to yield methyl phenyl urea.

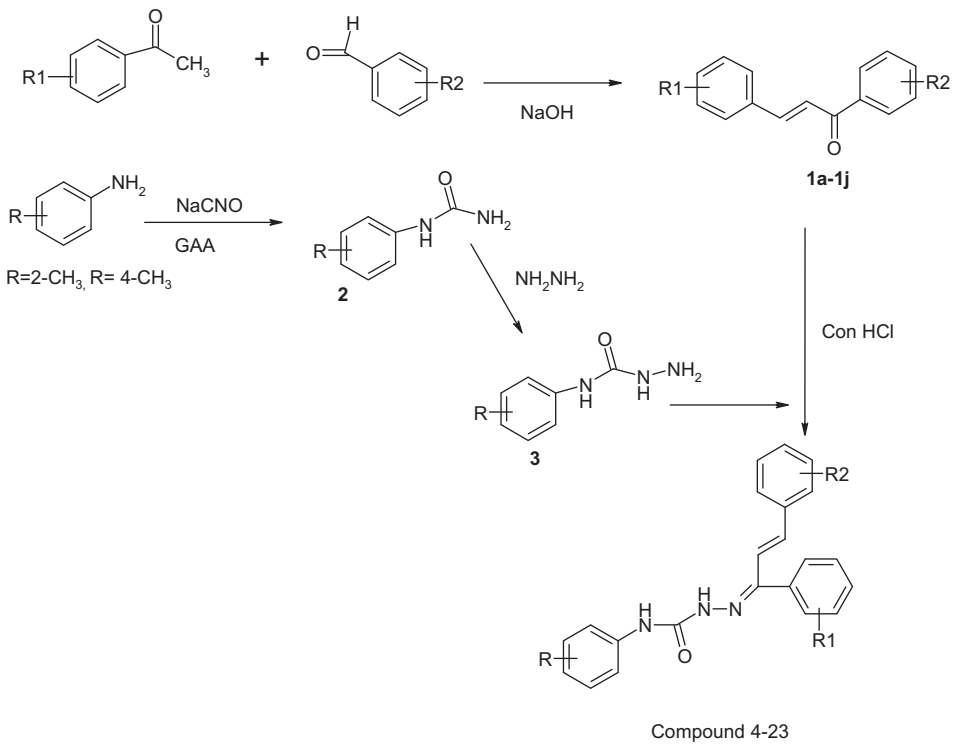

Figure 3 Synthetic scheme for synthesizing the Chalconesemicarbazone compounds.

Table 1 Physicochemical properties of chalcone derivatives.

Comp. no.	R_1	R_2	Molecular formula	Mp (°C)	Yield (%)	Rf value
1a	H	H	$C_{15}H_{12}O_2$	89	85	0.80
1b	H	4″-OH	$C_{15}H_{12}O_3$	164	85	0.83
1c	H	4″-OCH_3	$C_{16}H_{14}O_3$	135	85	0.82
1d	H	4″-$N(CH_3)_2$	$C_{17}H_{17}NO_2$	155	85	0.78
1e	4′-OH	6″-OH	$C_{15}H_{12}O_4$	216	90	0.85
1f	4′-OH	4″-$N(CH_3)_2$	$C_{17}H_{17}NO_3$	174	90	0.81
1g	H	6″-OH	$C_{15}H_{12}O_3$	166	85	0.86
1h	5′OH	6″-OH	$C_{15}H_{12}O_4$	218	85	0.84
1i	5′OH	4″-OH	$C_{15}H_{12}O_4$	208	85	0.87
1j	5′OH	4″-OCH_3	$C_{16}H_{14}O_4$	152	85	0.79

*2.1.3. Synthesis of substituted phenyl semicarbazide (**3**)*

Equimolar quantities (0.05 mol) of above phenyl urea (**2**) and hydrazine hydrate (2.5 ml) in ethanol were refluxed for 27 h with continuous stirring. The two-third volume of ethanol was distilled by vacuum distillation unit and then poured into ice. The resultant crude solid was filtered, washed with water and dried. The obtained solid was recrystallized with 50 ml of 90% alcohol.

2.1.4. General method for the synthesis of substituted phenyl chalconesemicarbazone

To a solution of above (**3**) (0.005 mol) in 25 ml of ethanol added an equimolar quantity of the appropriate chalcone derivative previously dissolved in ethanol. Then few drops of Con. hydrochloric acid was added and continuously stirred for 4–5 h.

The reaction mixture was poured into ice and precipitate, so obtained was filtered, washed with sodium acetate (0.005 mol, 0.41 g) in 2 ml water. The crude solid was dried and recrystallized with hot ethanol. The structures (Fig. 4) and physicochemical properties of the synthesized title compounds are given in Table 2.

*2.1.4.1. 1-[1-(2-Hydroxyphenyl)-3-phenylallylidene]-4-(2-methylphenyl)semicarbazide (**4**).* ^{1}H NMR (δ/ppm in $CDCl_3$): 2.12 (s, 3H, Ar–CH_3), 4.83 (s, 1H, 2-OH), 7.11–7.64 (m, J = 8.32 Hz, 12H, Ar–H) 7.7 (s, 1H, –CH═CH–), 7.9 (s, 1H, –CH═CH–), 8.34 (s, 1H, ArNH, D_2O exchangeable), 9.42 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3450 (NH), 3480 (–OH), 3300–3240 (CONH), 1670 (–CH═CH–), 1590 (C–N), 1616, 1558 (aromatic), 754, 697 (monosubstituted benzene); MS, m/z 370; Elemental analysis calculated/found (%) C (74.37/74.26), H (5.70/5.48), N (11.31/11.12).

*2.1.4.2. 1-[1-(2-Hydroxyphenyl)-3-(4-hydroxyphenyl)allylidene]-4-(2-methylphenyl)semicarbazide (**5**).* ^{1}H NMR (δ/ppm in $CDCl_3$): 2.18 (s, 3H, Ar–CH_3), 4.9 (s, 1H, 2-OH), 5.2 (s,

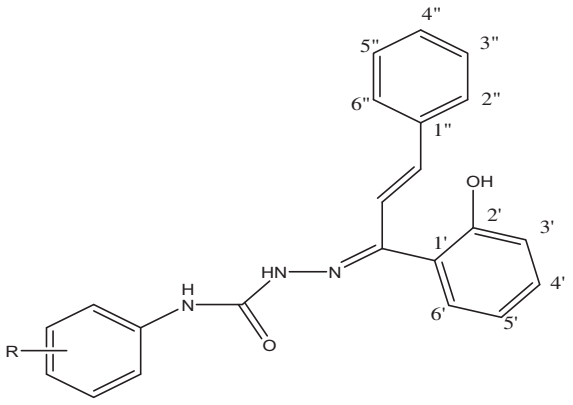

Figure 4 Structure of synthesized title compounds.

1H, 4-OH), 7.3–7.64 (m, J = 8.4 Hz, 11H, Ar–H) 7.8 (s, 1H, –CH═CH–), 8.0 (s, 1H, –CH═CH–), 8.44 (s, 1H, ArNH, D_2O exchangeable), 9.8 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3455 (NH), 3475 (–OH), 3310–3245 (CONH), 1675 (–CH═CH–), 1594 (C–N), 1615, 1556 (aromatic), 750, 695 (monosubstituted benzene); MS, m/z 386; Elemental analysis, cal/fou (%) C (71.30/71.24), H (5.46/5.35), N (10.85/10.47).

*2.1.4.3. 1-[1-(2-Hydroxyphenyl)-3-(4-methoxyphenyl)allylidene]-4-(2-methylphenyl)semicarbazide (**6**).* ^{1}H NMR (δ/ppm in $CDCl_3$): 2.16 (s, 3H, Ar–CH_3), 4.7 (s, 1H, 2-OH), 3.88 (s, 3H, 4-OCH_3), 7.12–7.85 (m, J = 8.3 Hz, 11H, Ar–H), 7.98 (s, 1H, –CH═CH–), 8.35 (s, 1H, –CH═CH–), 8.87 (s, 1H, ArNH, D_2O exchangeable), 9.86 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3458 (NH), 3478 (–OH), 3310–3243 (CONH), 1677 (–CH═CH–), 1587 (C–N), 1626, 1555 (aromatic), 758, 687 (monosubstituted benzene); MS, m/z 400; Elemental analysis cal/fou (%) C (71.80/71.57), H (5.77/5.48), N (10.47/10.36).

*2.1.4.4. 1-[1-(2,4-Dihydroxyphenyl)-3-(2-hydroxyphenyl)allylidene]-4-(2-methylphenyl)semicarbazide (**9**).* ^{1}H NMR (δ/ppm in $CDCl_3$): 2.48 (s, 3H, Ar–CH_3), 5.1 (s, 1H, 2-OH), 5.3 (s, 1H, 4-OH), 6.4 (s, 1H, 6-OH), 7.22–7.58 (m, J = 8.5 Hz, 10H, Ar–H) 7.88 (s, 1H, –CH═CH–), 8.4 (s, 1H, –CH═CH–), 8.77 (s, 1H, ArNH, D_2O exchangeable), 9.85 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3453 (NH), 3482 (–OH), 3314–3242 (CONH), 1667 (–CH═CH–), 1594 (C–N), 1618, 1552 (aromatic), 758, 687 (monosubstituted benzene); MS, m/z 402; Elemental analysis cal/fou (%) C (68.47/68.44), H (5.25/5.16), N (10.42/10.37).

*2.1.4.5. 1-[1-(2-Hydroxyphenyl)-3-(2-hydroxyphenyl)allylidene]-4-(2-methylphenyl)semicarbazide (**11**).* ^{1}H NMR (δ/ppm in $CDCl_3$): 2.24 (s, 3H, Ar–CH_3), 5.1 (s, 1H, 2-OH), 5.3 (s, 1H, 2, 4-OH), 7.2–7.78 (m, J = 8.35 Hz, 11H, Ar–H), 7.8 (s, 1H, –CH═CH–), 8.2 (s, 1H, –CH═CH–), 8.78 (s, 1H, ArNH, D_2O exchangeable), 9.84 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3462 (NH), 3488 (–OH), 3300–3240 (CONH), 1666 (–CH═CH–), 1593 (C–N), 1618, 1554 (aromatic), 753, 694 (monosubstituted benzene); MS, m/z 386; Elemental analysis cal/fou (%) C (71.30/71.17), H (5.46/5.37), N (10.85/10.66).

*2.1.4.6. 1-[1-(2,5-Dihydroxyphenyl)-3-(4-hydroxyphenyl)allylidene]-4-(2-methylphenyl)semicarbazide (**13**).* ^{1}H NMR (δ/ppm in $CDCl_3$): 2.16 (s, 3H, Ar–CH_3), 5.4 (s, 1H, 2-OH) 5.2 (s, 1H, 4-OH), 5.6 (s, 3H, 5-OH) 7.22–7.88 (m, J = 8.6 Hz, 10H, Ar–H), 7.84 (s, 1H, –CH═CH–), 8.4 (s, 1H, –CH═CH–), 8.82 (s, 1H, ArNH, D_2O exchangeable), 9.96 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3456 (NH), 3482 (–OH), 3310–3245 (CONH), 1667 (–CH═CH–), 1593 (C–N), 1615, 1552 (aromatic), 755, 693 (monosubstituted benzene); MS, m/z 402; Elemental analysis cal/fou (%) C (68.47/68.28), H (5.25/5.17), N (10.42/10.08).

*2.1.4.7. 1-[1-(2-Hydroxyphenyl)-3-phenylallylidene]-4-(4-methylphenyl)semicarbazide (**14**).* ^{1}H NMR (δ/ppm in $CDCl_3$): 2.15

Table 2 Physicochemical data of methyl semicarbazones.

Comp. no.	R	R_1	R_2	Yield (%)	Mol Wt.	Mol formula	Mp (°C)	Rf value
4	2-CH_3	H	H	57	371	$C_{23}H_{21}N_3O_2$	150	0.78
5	2-CH_3	H	4″-OH	66	387	$C_{23}H_{21}N_3O_3$	145	0.71
6	2-CH_3	H	4″-OCH_3	65	401	$C_{24}H_{23}N_3O_3$	135	0.65
7	2-CH_3	H	4″-$N(CH_3)_2$	58	414	$C_{25}H_{26}N_4O_2$	148	0.57
8	2-CH_3	4-OH	6″-OH	57	403	$C_{23}H_{21}N_3O_4$	142	0.60
9	2-CH_3	4-OH	4″-$N(CH_3)_2$	50	430	$C_{25}H_{26}N_4O_3$	160	0.67
10	2-CH_3	H	6″-OH	63	387	$C_{23}H_{21}N_3O_3$	140	0.55
11	2-CH_3	5-OH	6″-OH	61	403	$C_{23}H_{21}N_3O_4$	135	0.63
12	2-CH_3	5-OH	4″-OH	56	403	$C_{23}H_{21}N_3O_4$	120	0.69
13	2-CH_3	5-OH	4″-OCH_3	57	417	$C_{24}H_{23}N_3O_4$	126	0.51
14	4-CH_3	H	H	52	371	$C_{23}H_{21}N_3O_2$	206	0.53
15	4-CH_3	H	4″-OH	65	387	$C_{23}H_{21}N_3O_3$	188	0.63
16	4-CH_3	H	4″-OCH_3	63	401	$C_{24}H_{23}N_3O_3$	204	0.70
17	4-CH_3	H	4″-$N(CH_3)_2$	64	414	$C_{25}H_{26}N_4O_2$	195	0.62
18	4-CH_3	4-OH	6″-OH	55	403	$C_{23}H_{21}N_3O_4$	178	0.58
19	4-CH_3	4-OH	4″-$N(CH_3)_2$	56	430	$C_{25}H_{26}N_4O_3$	185	0.66
20	4-CH_3	H	6″-OH	54	387	$C_{23}H_{21}N_3O_3$	180	0.69
21	4-CH_3	5-OH	6″-OH	67	403	$C_{23}H_{21}N_3O_4$	183	0.54
22	4-CH_3	5-OH	4″-OH	50	403	$C_{23}H_{21}N_3O_4$	165	0.59
23	4-CH_3	5-OH	4″-OCH_3	56	417	$C_{24}H_{23}N_3O_4$	172	0.77

(s, 3H, Ar–CH_3), 4.82 (s, 1H, 2-OH), 7.22–7.64 (m, J = 8.3 Hz, 12H, Ar–H), 7.72 (s, 1H, –CH═CH–), 7.89 (s, 1H, –CH═CH–), 8.33 (s, 1H, ArNH, D_2O exchangeable), 9.38 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3452 (NH), 3485 (–OH), 3300–3243 (CONH), 1668(–CH═CH–), 1591 (C–N), 1613, 1548 (aromatic), 753, 695 (monosubstituted benzene); MS, m/z 370; Elemental analysis calculated/found (%) C (74.37/74.13), H (5.70/5.47), N (11.31/10.98).

*2.1.4.8. 1-[1-(2-Hydroxyphenyl)-3-(4-hydroxyphenyl)allylidene]-4-(4-methylphenyl)semicarbazide (**15**).* ^{1}H NMR (δ/ppm in $CDCl_3$): 2.17 (s, 3H, Ar–CH_3), 4.91 (s, 1H, 2-OH), 5.3 (s, 1H, 4-OH), 7.3–7.68 (m, J = 8.32 Hz, 11H, Ar–H), 7.79 (s, 1H, –CH═CH–), 8.1 (s, 1H, –CH═CH–), 8.42 (s, 1H, ArNH, D_2O exchangeable), 9.85 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3449 (NH), 3471 (–OH), 3318–3245 (CONH), 1676 (–CH═CH–), 1593 (C–N), 1618, 1559 (aromatic), 751, 696 (monosubstituted benzene); MS, m/z 386; Elemental analysis, cal/fou (%) C (71.30/71.25), H (5.46/5.33), N (10.85/10.58).

*2.1.4.9. 1-[1-(2-Hydroxyphenyl)-3-(4-methoxyphenyl)allylidene]-4-(4-methylphenyl)semicarbazide (**16**).* ^{1}H NMR (δ/ppm in $CDCl_3$): 2.19 (s, 3H, Ar–CH_3), 4.74 (s, 1H, 2-OH), 3.83 (s, 3H, 4-OCH_3), 7.12–7.85 (m, J = 8.3 Hz, 11H, Ar–H), 7.95 (s, 1H, –CH═CH–), 8.36 (s, 1H, –CH═CH–), 8.89 (s, 1H, ArNH, D_2O exchangeable), 9.86 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3454 (NH), 3479 (–OH), 3310–3243 (CONH), 1672 (–CH═CH–), 1589 (C–N), 1624, 1556 (aromatic), 753, 687 (monosubstituted benzene); MS, m/z 400; Elemental analysis cal/fou (%) C (71.80/71.68), H (5.77/5.67), N (10.47/10.33).

*2.1.4.10. 1-[1-(2,4-Dihydroxyphenyl)-3-(2-hydroxyphenyl)allylidene]-4-(4-methylphenyl)semicarbazide (**18**).* ^{1}H NMR (δ/ppm in $CDCl_3$): 2.38 (s, 3H, Ar–CH_3), 5.22 (s, 1H, 2-OH), 5.37 (s, 1H, 4-OH), 6.43 (s, 1H, 6-OH), 7.22–7.58 (m, J = 8.32 Hz, 10H, Ar–H) 7.89 (s, 1H, –CH═CH–), 8.421 (s, 1H, –CH═CH–), 8.77 (s, 1H, ArNH, D_2O exchangeable), 9.86 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3456 (NH), 3482 (–OH), 3314–3242 (CONH), 1665 (–CH═CH–), 1598 (C–N), 1616, 1554 (aromatic), 752, 689 (monosubstituted benzene); MS, m/z 402; Elemental analysis cal/fou (%) C (68.47/68.44), H (5.25/5.21), N (10.42/10.33).

*2.1.4.11. 1-[1-(2-Hydroxyphenyl)-3-(2-hydroxyphenyl)allylidene]-4-(4-methylphenyl)semicarbazide (**20**).* ^{1}H NMR (δ/ppm in $CDCl_3$): 2.25 (s, 3H, Ar–CH_3), 5.14 (s, 1H, 2-OH), 5.29 (s, 1H, 2, 4-OH), 7.2–7.77 (m, J = 8.3 Hz, 11H, Ar–H), 7.82 (s, 1H, –CH═CH–), 8.2 (s, 1H, –CH═CH–), 8.77 (s, 1H, ArNH, D_2 Oexchangeable), 9.87 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3462 (NH), 3488 (–OH), 3300–3240 (CONH), 1666 (–CH═CH–), 1593 (C–N), 1618, 1554 (aromatic), 753, 694 (monosubstituted benzene); MS, m/z 386; Elemental analysis cal/fou (%) C (71.30/71.13), H (5.46/5.42), N (10.85/10.72).

*2.1.4.12. 1-[1-(2,5-Dihydroxyphenyl)-3-(4-hydroxyphenyl)allylidene]-4-(4-methylphenyl)semicarbazide (**22**).* ^{1}H NMR (δ/ppm in $CDCl_3$): 2.17 (s, 3H, Ar–CH_3), 5.45 (s, 1H, 2-OH) 5.22 (s, 1H, 4-OH), 5.61 (s, 3H, 5-OH) 7.22–7.88 (m, J = 8.6 Hz, 10H, Ar–H), 7.85 (s, 1H, –CH═CH–), 8.4 (s, 1H, –CH═CH–), 8.82 (s, 1H, ArNH, D_2O exchangeable), 9.98 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3458 (NH), 3483 (–OH), 3311–3246 (CONH), 1669 (–CH═CH–), 1595 (C–N), 1617, 1555 (aromatic), 756, 699 (monosubstituted benzene); MS, m/z 402; Elemental analysis cal/fou (%) C (68.47/68.33), H (5.25/5.13), N (10.42/10.31).

2.2. Reducing power assay

The antioxidant activity of chalconesemicarbazones was determined by the method of reducing power assay (Oyaizu, 1986). Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

$$\text{Potassium ferricyanide} + \text{Ferric chloride} \xrightarrow{\text{Antioxidant}} \text{Potassium ferrocyanide} + \text{ferrous chloride}$$

One millilitre of test sample solution (20 μg/ml) was mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50 °C for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. Ascorbic acid (20 μg/ml) was used as standard. A blank was prepared without adding standard or test compound. Increased absorbance of the reaction mixture indicates increase in reducing power (Khanam et al., 2004).

The percent increase in reducing power was calculated using the following equation

$$\text{Increase in reducing power } (\%) = \frac{\text{A test} - \text{A blank}}{\text{A blank}} \times 100$$

where 'A test' is absorbance of test solution; 'A blank' is absorbance of blank.

3. Results and discussion

The antioxidant activity of the synthesized chalcone semicarbazones was evaluated using reducing power assay. The results of anti-oxidant screening were depicted in Table 3 and Fig. 5. Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm (Oyaizu, 1986; Khanam et al., 2004).

As from the tables it could be seen that most of the compounds showed significant antioxidant activity. The highest reducing activity observed in compound **18** is probably due to the presence of hydroxyl group in the acetophenic and aldehydic moiety of chalcone. The order of activity regarding substitution on chalconyl group is OH > OCH_3 > $(CH_3)_2$–N > H (Venkateswarlu et al., 2006; Singh et al., 2007; Flynn et al., 1991).

When the observed results were compared, it was observed that the 4 methyl substituted compounds showed more reducing power in comparison to the 2 methyl substituted com-

Table 3 Antioxidant activity of chalcone semicarbazones by reducing power assay.

Compounds	Absorbance (mean ± SD; 700 nm)	% Increase in reducing power
Control	0.0952 ± 0.0002	–
Standard	0.187 ± 0.0036[a]	96.43
4	0.1102 ± 0.00026[a]	15.76
5	0.1429 ± 0.0027[a]	50.1
6	0.1402 ± 0.00056[a]	47.27
7	0.1189 ± 0.00036[a]	24.89
8	0.1803 ± 0.0003[a]	89.39
9	0.1236 ± 0.0005[a]	29.83
10	0.1421 ± 0.00026[a]	49.26
11	0.1763 ± 0.00068[a]	85.19
12	0.1668 ± 0.00036[a]	75.21
13	0.1464 ± 0.00046[a]	53.78
14	0.1179 ± 0.0002[a]	23.84
15	0.1437 ± 0.00077[a]	50.94
16	0.1411 ± 0.0001[a]	48.21
17	0.1208 ± 0.00089[a]	26.89
18	0.19 ± 0.00199[a]	99.58
19	0.1281 ± 0.00011[a]	34.56
20	0.1274 ± 0.0005[a]	33.82
21	0.1891 ± 0.00044[a]	98.63
22	0.182 ± 0.00066[a]	91.18
23	0.1497 ± 0.0006[a]	57.25

[a] $P < 0.001$ compared to control and standard respectively. One way ANOVA followed by Turkey test.

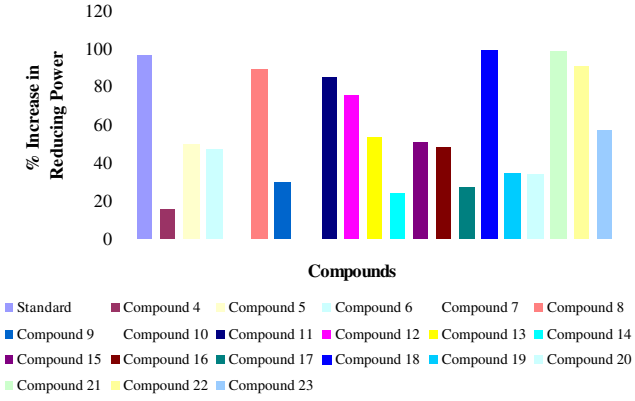

Figure 5 Antioxidant activity of chalcone semicarbozones by reducing power assay.

pounds. The substitution with different substituent on the phenyl of the aldehydic and acetophenic group of chalcone moiety plays an important role in reducing potential of the compounds.

When the phenyl group of aldehydic and acetophenic moiety of chalcone is substituted with –OH group (compounds **8**, **11**, **12**, **18**, **21**, **22**) the compounds exhibited better activity in comparison to substitution with the other groups like p-dimethyl amino groups (compounds **7**, **17**, **19**), methoxy group (compounds **6**, **13**, **16**, **23**) which may be due to more reducing potential. Hydroxyl substitution on both moieties of chalcone has more scavenging effect than substitution on any one moiety (Venkateswarlu et al., 2006; Singh et al., 2007; Flynn et al., 1991). It was found that 6 hydroxyl substitution is more favorable than 4 or 5 hydroxyl substitution in the aldehydic moiety for antioxidant activity.

Methoxy substitution in the aldehydic moiety of chalcone also favors antioxidant activity (compounds **7**–**10**). It shows more reducing potential with hydroxyl substitution in the acetophenic moiety than unsubstituted ones (Madhavi et al., 2010).

Among the synthesized compounds, compounds **18** and **21** showed the better or comparable activity in comparison to the standard drug while the other compounds showed moderate reductive potential. In case of bulkier substitution (compounds **7**, **9**, **17**, **19**), the substitution increases the activity which may be due to the high electronegativity. The compounds with no substitution (compounds **4** and **14**) or less substitution showed very less reducing power in comparison to the substituted compounds due to lesser electronegativity (Venkateswarlu et al., 2006; Singh et al., 2007; Flynn et al., 1991; Rajasekaran et al., 2010; Allegra et al., 2003).

In summary, most of the synthesized compounds were potential leads for antioxidant activity. On the bases of observed results, it may be concluded that the substitution favors the activity, but the lengthening of carbon chain may also disfavor the activity, may be due to the lesser electronegativity. The hydroxyl and methoxy substitution increases the activity of the compounds, which may be due to increased electronegativity.

References

Allegra, M., Reiter, R.J., Tan, D.X., Gentile, C., Tesoriere, L., Livrea, M.A., 2003. The chemistry of melatonin's interaction with reactive species. J. Pineal. Res. 34, 1–10.

Dutta, S., Padhye, S., Priyadarsini, K.I., Newton, C., 2005. Antioxidant and antiproliferative activity of curcumin semicarbazone. Bioorg. Med. Chem. Lett. 15, 2738–2744.

Flynn, D.L., Belliotti, T.R., Boctor, A.M., Connor, D.T., Kostlan, C.R., Nies, D.E., Ortwine, D.F., Schrier, J.D., Sircar, J.C., 1991. Styrylpyrazoles, styryisoxazoles and styrylisothiazoles: Novel 5′-lipoxygenase and cyclooxygenase inhibitors. J. Med. Chem. 34, 518–525.

Khanam, S., Shivaprasada, H.N., Devi, K., 2004. In vitro antioxidant screening models: a review. Ind. J. Pharm. Edu. Res. 38 (4), 180–183.

Madhavi, K., Bharathi, K., Prasad, K.V.S.R.G., 2010. Synthesis and evaluation of 3-methyl-4-nitro-5-(substitutedstyryl) isoxazoles for antioxidant and anti-inflammatory activities. Res. J. Pharm. Biol. Chem. Sci. 1 (4), 1073–1082.

Nickels, W., 2003. The mystery of non-classical protein secretion. Eur. J. Biochem. 270, 2109–2119.

Oyaizu, M., 1986. Studies on product of browning reaction prepared from glucose amine. Jpn. J. Nutr. 44, 307–315.

Rajasekaran, S., Rao, G.K., Pai, P.N.S., Sodhi, G.S., 2010. Synthesis, antibacterial and invitro antioxidant activity of 2,3-substituted quinazolin-4(3H)-ones. J. Chem. Pharm. Res. 2 (1), 482–488.

Setiadi, D.H., Chass, G.A., Torday, L.L., Varro, A., Papp, J.G., 2003. Vitamin E models: Can the antioxidants and pro-antioxidant dichotomy of α-tocopherol be related to ionic ring closing and radical ring opening redox reactions. J. Mol. Struct. 620, 93–106.

Singh, D., Mohan, S., Sharma, P.C., Sarvanan, J., 2007. Synthesis and evaluation of some novel piperidino thiophenes as potential antioxidant and anti-inflammatory agents. Acta Pharm. Sci. 49, 29–38.

Venkateswarlu, S., Ramchandra, M.S., Krishnaraju, A.V., Trimurtulu, G., Subbaraju, G.V., 2006. Antioxidant and antimicrobial activity of polyhydroxycinnamic acid ester derivatives. Ind. J. Chem. 45B, 252–257.