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Nitric oxide scavenging activity of synthesized methyl semicarbazone derivatives

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ABSTRACT

In present study, a series of chalconesemicarbazones was synthesized and evaluated for their nitric oxide scavenging activity. Most of the compounds were found to be potent nitric oxide scavenger. Nitric oxide plays an important role in various pathological and xenotoxic effects so nitric oxide scavenger may have protective role in these pathological conditions. Based on the results of an anti-oxidant study, 1-[1-(2-hydroxyphenyl)-3-(4-methoxyphenyl)allylidene]-4-(2-methylphenyl) semicarbazide (Compound 6) was the most active compound. It was found that methoxy and hydroxyl substituted chalconesemicarbazones were potent nitric oxide scavenger and unsubstituted compound showed very less activity.

Keywords: Chalcones, Anti-oxidant, Semicarbazones, Nitric oxide scavenging.

INTRODUCTION

NO is an important chemical mediator generated by endothelial cells, macrophages, neurons and is involved in the regulation of various physiological processes [1]. Excess concentration of NO is associated with several diseases [2-3]. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions which acts as free radicals [4]. Nitric oxide can react rapidly in the intracellular environment to form nitrate, nitrite and s-nitrosothiols. These metabolites play a key role in mediating many xenotoxic effects such as DNA damage. NO causes DNA damage via peroxynitrite.

Diazotization takes place between nitrite and sulphanilamide, this diazotized product is coupled with naphthylene diamine to form chromophore, which is reduced by antioxidant when measured at 546nm [5]. Nitric oxide (NO) was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent. SNP in aqueous solution at physiological pH spontaneously generates NO [6-7] which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess Reagent. Scavengers of NO compete with oxygen leading to reduced production of NO [7-8].

MATERIALS AND METHODS**Chemistry**

Chalconesemicarbazones were synthesized according to synthetic scheme as shown in figure 1. 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 Bruker 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 D₂O. 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.

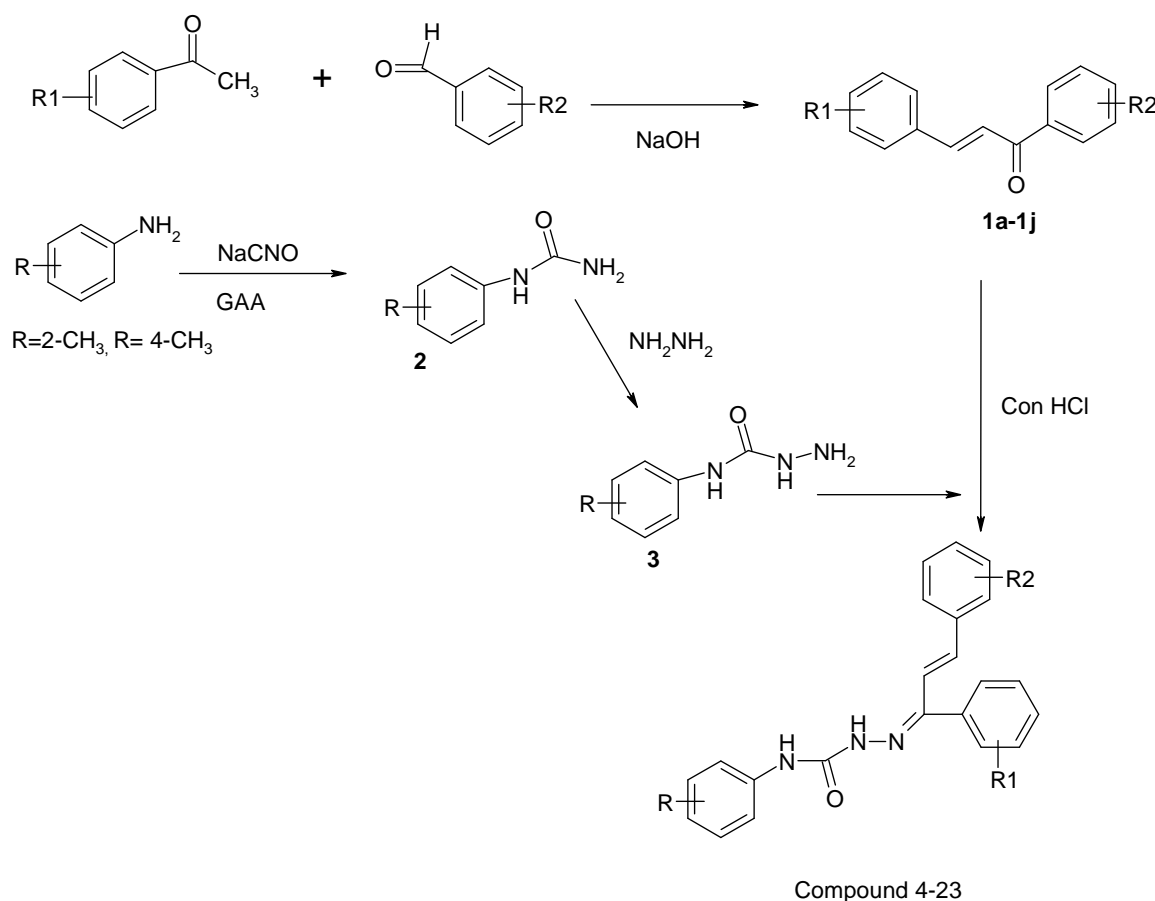


Figure 1: synthetic scheme for synthesizing the title compounds

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.

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

Synthesis of methyl phenyl urea (2)

Substituted aniline (0.1mol) 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.

Synthesis of substituted phenyl semicarbazide (3)

Equimolar quantities (0.05mol) 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.

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 hrs.

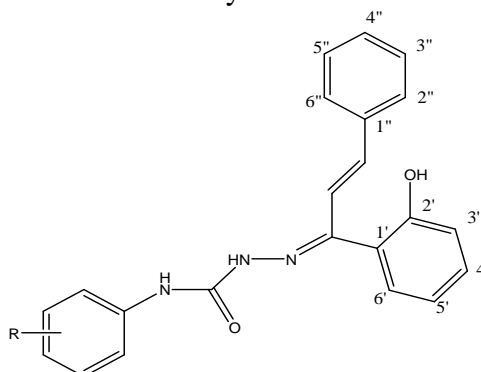


Figure 2: Structure of synthesized title compounds

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

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 ₃ O ₂	150	0.78
5	2-CH ₃	H	4''-OH	66	387	C ₂₃ H ₂₁ N ₃ O ₃	145	0.71
6	2-CH ₃	H	4''-OCH ₃	65	401	C ₂₄ H ₂₃ N ₃ O ₃	135	0.65
7	2-CH ₃	H	4''-N(CH ₃) ₂	58	414	C ₂₅ H ₂₆ N ₄ O ₂	148	0.57
8	2-CH ₃	4-OH	6''-OH	57	403	C ₂₃ H ₂₁ N ₃ O ₄	142	0.60
9	2-CH ₃	4-OH	4''-N(CH ₃) ₂	50	430	C ₂₅ H ₂₆ N ₄ O ₃	160	0.67
10	2-CH ₃	H	6''-OH	63	387	C ₂₃ H ₂₁ N ₃ O ₃	140	0.55
11	2-CH ₃	5-OH	6''-OH	61	403	C ₂₃ H ₂₁ N ₃ O ₄	135	0.63
12	2-CH ₃	5-OH	4''-OH	56	403	C ₂₃ H ₂₁ N ₃ O ₄	120	0.69
13	2-CH ₃	5-OH	4''-OCH ₃	57	417	C ₂₄ H ₂₃ N ₃ O ₄	126	0.51
14	4-CH ₃	H	H	52	371	C ₂₃ H ₂₁ N ₃ O ₂	206	0.53
15	4-CH ₃	H	4''-OH	65	387	C ₂₃ H ₂₁ N ₃ O ₃	188	0.63
16	4-CH ₃	H	4''-OCH ₃	63	401	C ₂₄ H ₂₃ N ₃ O ₃	204	0.70
17	4-CH ₃	H	4''-N(CH ₃) ₂	64	414	C ₂₅ H ₂₆ N ₄ O ₂	195	0.62
18	4-CH ₃	4-OH	6''-OH	55	403	C ₂₃ H ₂₁ N ₃ O ₄	178	0.58
19	4-CH ₃	4-OH	4''-N(CH ₃) ₂	56	430	C ₂₅ H ₂₆ N ₄ O ₃	185	0.66
20	4-CH ₃	H	6''-OH	54	387	C ₂₃ H ₂₁ N ₃ O ₃	180	0.69
21	4-CH ₃	5-OH	6''-OH	67	403	C ₂₃ H ₂₁ N ₃ O ₄	183	0.54
22	4-CH ₃	5-OH	4''-OH	50	403	C ₂₃ H ₂₁ N ₃ O ₄	165	0.59
23	4-CH ₃	5-OH	4''-OCH ₃	56	417	C ₂₄ H ₂₃ N ₃ O ₄	172	0.77

1-[1-(2-hydroxyphenyl)-3-phenylallylidene]-4-(2-methylphenyl) semicarbazide (4)

¹H-NMR (δ/ppm in CDCl₃): 2.12 (s, 3H, Ar-CH₃), 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, D₂O exchangeable), 9.42 (s, 1H, CONH, D₂O 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).

1-[1-(2-hydroxyphenyl)-3-(4-hydroxyphenyl)allylidene]-4-(2-methylphenyl) semicarbazide (5)

¹H-NMR (δ/ppm in CDCl₃): 2.18 (s, 3H, Ar-CH₃), 4.9 (s, 1H, 2-OH), 5.2 (s, 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, D₂O exchangeable), 9.8 (s, 1H, CONH, D₂O 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).

1-[1-(2-hydroxyphenyl)-3-(4-methoxyphenyl)allylidene]-4-(2-methylphenyl) semicarbazide (6)

¹H-NMR (δ /ppm in CDCl₃): 2.16 (s, 3H, Ar-CH₃), 4.7 (s, 1H, 2-OH), 3.88 (s, 3H, 4-OCH₃), 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, D₂O exchangeable), 9.86 (s, 1H, CONH, D₂O 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).

1-[1-(2,4-dihydroxyphenyl)-3-(2-hydroxyphenyl)allylidene]-4-(2-methylphenyl) semicarbazide (9)

¹H-NMR (δ /ppm in CDCl₃): 2.48 (s, 3H, Ar-CH₃), 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, D₂O exchangeable), 9.85 (s, 1H, CONH, D₂O 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).

1-[1-(2-hydroxyphenyl)-3-(2-hydroxyphenyl)allylidene]-4-(2-methylphenyl) semicarbazide (11)

¹H-NMR (δ /ppm in CDCl₃): 2.24 (s, 3H, Ar-CH₃), 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, D₂O exchangeable), 9.84 (s, 1H, CONH, D₂O 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).

1-[1-(2,5-dihydroxyphenyl)-3-(4-hydroxyphenyl)allylidene]-4-(2-methylphenyl) semicarbazide (13)

¹H-NMR (δ /ppm in CDCl₃): 2.16 (s, 3H, Ar-CH₃), 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, D₂O exchangeable), 9.96 (s, 1H, CONH, D₂O 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).

1-[1-(2-hydroxyphenyl)-3-phenylallylidene]-4-(4-methylphenyl)semicarbazide (14)

¹H-NMR (δ /ppm in CDCl₃): 2.15 (s, 3H, Ar-CH₃), 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, D₂O exchangeable), 9.38 (s, 1H, CONH, D₂O 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).

1-[1-(2-hydroxyphenyl)-3-(4-hydroxyphenyl)allylidene]-4-(4-methylphenyl) semicarbazide (15)

¹H-NMR (δ /ppm in CDCl₃): 2.17 (s, 3H, Ar-CH₃), 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, D₂O exchangeable), 9.85 (s, 1H, CONH, D₂O 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).

1-[1-(2-hydroxyphenyl)-3-(4-methoxyphenyl)allylidene]-4-(4-methylphenyl) semicarbazide (16)

¹H-NMR (δ /ppm in CDCl₃): 2.19 (s, 3H, Ar-CH₃), 4.74 (s, 1H, 2-OH), 3.83 (s, 3H, 4-OCH₃), 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, D₂O exchangeable), 9.86 (s, 1H, CONH, D₂O 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).

1-[1-(2,4-dihydroxyphenyl)-3-(2-hydroxyphenyl)allylidene]-4-(4-methylphenyl) semicarbazide (18)

¹H-NMR (δ/ppm in CDCl₃): 2.38 (s, 3H, Ar-CH₃), 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, D₂O exchangeable), 9.86 (s, 1H, CONH, D₂O 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).

1-[1-(2-hydroxyphenyl)-3-(2-hydroxyphenyl)allylidene]-4-(4-methylphenyl) semicarbazide (20)

¹H-NMR (δ/ppm in CDCl₃): 2.25 (s, 3H, Ar-CH₃), 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, D₂O exchangeable), 9.87 (s, 1H, CONH, D₂O 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).

1-[1-(2,5-dihydroxyphenyl)-3-(4-hydroxyphenyl)allylidene]-4-(4-methylphenyl) semicarbazide (22)

¹H-NMR (δ/ppm in CDCl₃): 2.17 (s, 3H, Ar-CH₃), 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, D₂O exchangeable), 9.98 (s, 1H, CONH, D₂O 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).

Nitric oxide scavenging assay

0.5ml of Sodium nitropruside (10mM) was mixed with 0.5ml of chalcone semicarbazone (40µg/ml) dissolved in methanol and incubated at room temperature for 150 min. The same reaction mixture without the sample but with equivalent amount of phosphate buffer saline served as control. After, the incubation period, 0.5ml of Griess reagent (1% sulphanilamide, 2% H₃PO₄ and 0.1% naphthylethylenediamine dihydrochloride) was added. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine was read at 546nm. While 0.5ml of Ascorbic acid (40µg/ml) was used as positive control. Percent inhibition was determined by comparing the results of the test and control samples.

$$\text{Nitric Oxide scavenged (\%)} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

Where, *A*_{control} = Absorbance of control reaction and *A*_{test} = Absorbance in the presence of the samples of test compounds.

RESULTS AND DISCUSSION

The antioxidant activity of the synthesized chalcone semicarbazones was evaluated using nitric oxide scavenging assay. The results of anti-oxidant screening were depicted in Table 3 and figure 3. As from the tables it could be seen that most of the compounds showed significant nitric oxide scavenging activity. The highest scavenging activity observed in compound 6 is probably due to the presence of methoxy group in the aldehydic moiety of

chalcone. The order of activity regarding substitution on aldehydic moiety of the chalconyl group is $\text{OCH}_3 > \text{OH} > \text{H} > (\text{CH}_3)_2\text{-N}$.

Table 3: Antioxidant activity of chalcone semicarbazones by nitric oxide scavenging assay

Compounds	Absorbance (mean±S.D.; 546 nm)	% Nitric oxide scavenging
Control	0.932±0.0089	-----
Standard	0.216±0.0128 ^a	76.82
Compound 4	0.761±0.027 ^{a,b}	18.35
Compound 5	0.469±0.0165 ^{a,b}	49.68
Compound 6	0.169±0.0053 ^a	81.87
Compound 7	0.821±0.018 ^{a,b}	11.91
Compound 8	0.407±0.0123 ^{a,b}	56.33
Compound 9	0.834±0.0108 ^{a,b}	10.51
Compound 10	0.465±0.0147 ^{a,b}	50.11
Compound 11	0.343±0.0121 ^{a,b}	63.197
Compound 12	0.413±0.0182 ^{a,b}	55.69
Compound 13	0.256±0.007 ^a	72.53
Compound 14	0.783±0.0181 ^{a,b}	15.99
Compound 15	0.587±0.0082 ^{a,b}	37.02
Compound 16	0.212±0.0142 ^a	77.25
Compound 17	0.891±0.0193 ^b	4.399
Compound 18	0.528±0.014 ^{a,b}	43.35
Compound 19	0.912±0.003 ^b	2.15
Compound 20	0.561±0.003 ^{a,b}	39.81
Compound 21	0.434±0.0026 ^{a,b}	53.43
Compound 22	0.519±0.0092 ^{a,b}	44.31
Compound 23	0.309±0.0082 ^{a,b}	66.84

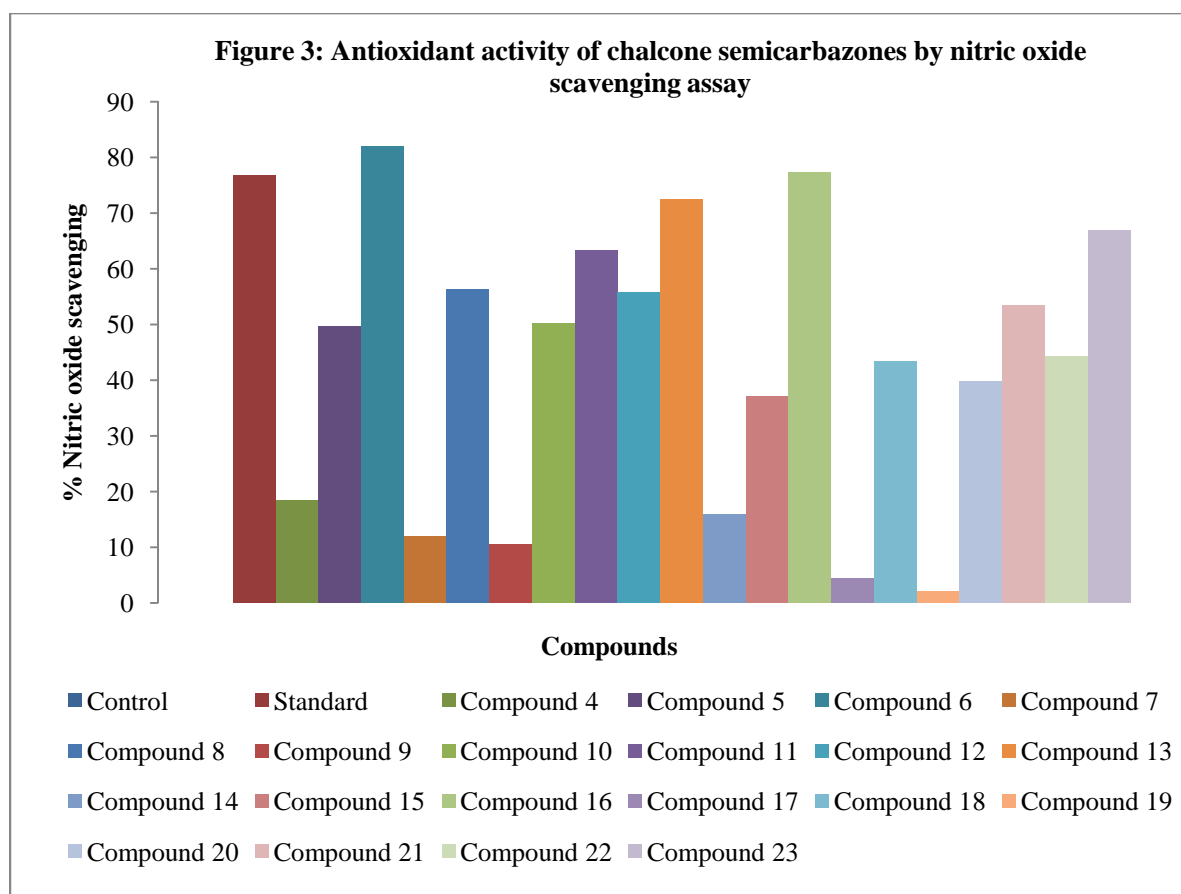
^{a,b} *P* < 0.001 compared to control and standard respectively. One way ANOVA followed by Turkey test

When the observed results compared, it observed that the 2 methyl substituted compounds exhibited better nitric oxide scavenging effect in comparison to the 4 methyl substituted compounds. The substitution with different substituent on the phenyl of the aldehydic and acetophenic group of chalcone moiety plays an important role in the scavenging of nitric oxide free radical.

When the phenyl group of aldehydic moiety of chalcone is substituted with $-\text{OCH}_3$ group (Compound 6, 16) without acetophenic substitution the compounds exhibited better activity in comparison to substitution with the other groups like p-dimethyl amino groups (compound 7, 17, 19), Hydroxyl group (compound 5, 8, 10, 11, 12, 21, 22).

When the phenyl group of aldehydic and acetophenic moiety of chalcone is substituted with $-\text{OH}$ group (Compound 8, 11, 12, 18, 21, 22) the compounds exhibited good scavenging activity. 5 Hydroxyl substitution on acetophenic moiety showed better nitric oxide scavenging effect than 4 or 6 hydroxyl substitution similarly 6 Hydroxyl substitution on aldehydic moiety exhibited better nitric oxide scavenging effect than 4 or 5 hydroxyl substitution.

Among the synthesized compounds, compound 6, 13, 16 showed the better or comparable activity in comparison to the standard drug while the other compounds showed moderate antioxidant activity. The bulkier substitution (compound 7, 9, 17, 19) does not favor scavenging of nitric oxide. The compounds with no substitution (compound 4, 14) or less substitution were showed very less reducing power in comparison to the substituted compounds.



In summary, most of the synthesized compounds were potential lead for antioxidant activity. On the bases of observed results, it may be concluded that the substitution favors the activity, but the bulkier substitution disfavors the scavenging activity. The methoxy and hydroxyl substitution increases the nitric oxide scavenging activity of the compounds.

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