Synthesis, Characterization and Anti-Inflammatory Activity of Some Novel Chalconesemicarbazones

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Summary

A series of chalconesemicarbazones was synthesized and evaluated for their anti-inflammatory activities. Most of the compounds were found to be more or comparable potent than the reference standard drug in carrageenan-induced rat paw edema test. Based on the results of an anti-inflammatory study, 1-[1-(2-hydroxyphenyl)-3-(2-hydroxyphenyl)allylidene]-4-(2-methylphenyl) semicarbazide **(**11) was the most active compound.

Keywords: Chalcones, Anti-inflammatory activity, Semicarbazones

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Introduction

Non steroidal anti-inflammatory drugs (NSAID's) are widely used in the treatment of pain and inflammation. NSAID's reduce the pain and swelling associated with arthritis by blocking the metabolism of arachidonic acid (AA) through the enzyme cyclooxygenase (COX) and thereby the production of prostaglandins, e. g. PGE2, which sensitizes nociceptors at nerve fiber terminals [1, 2]. There are several reports about the synthesis and pharmacological evaluation of new bioactive N-aroylarylhydrazones acting at the AA cascade enzyme level [3–10] and chalcones are also having anti-inflammatory activity. As a part of our ongoing research program [11–13] to find novel anti-inflammatory compounds, herein, we have fused these both active moiety and design a scheme for synthesizing these. The anti-inflammatory activity of synthesized compounds was performed.

Experimental Section

Chemistry

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.

Synthesis of substituted chalcone derivatives

Substituted benzaldehydes (0.012mol) were added to a mixture of substituted acetophenones (0.01mol) 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 structure (figure 2) and physicochemical properties of the synthesized chalcone derivatives are given in table 1.

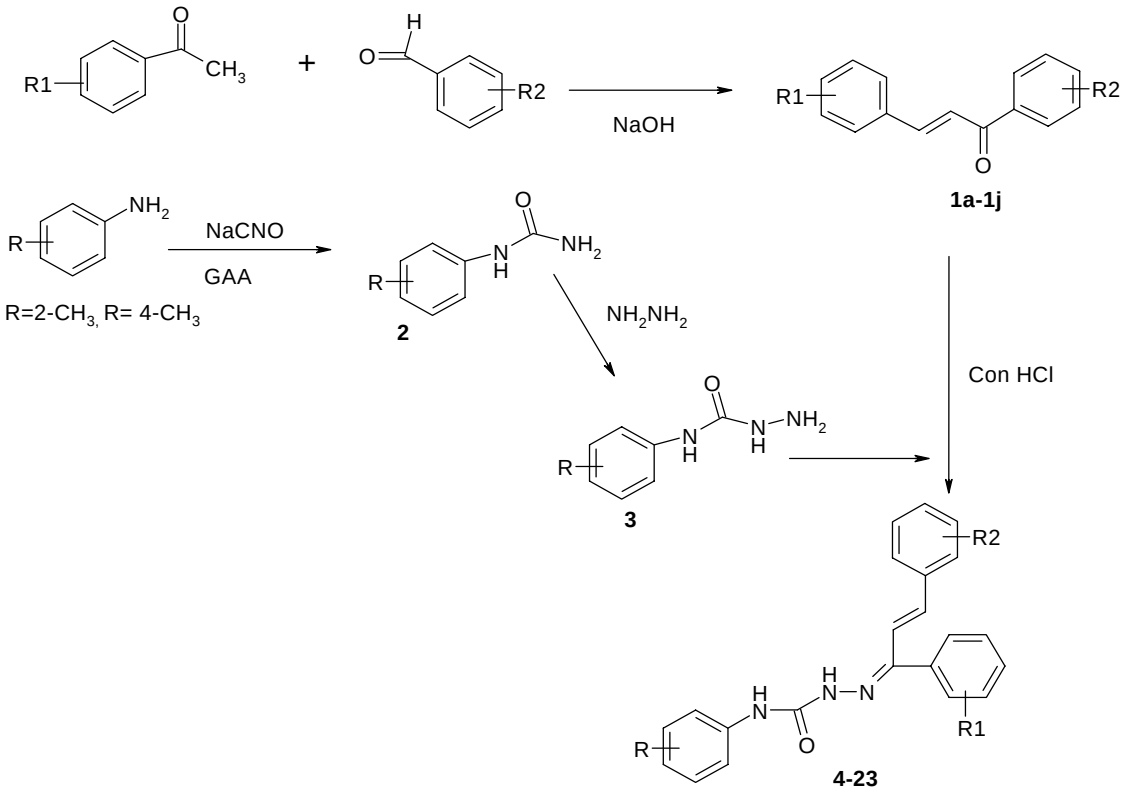

Figure 1: ***synthetic scheme for synthesizing the title compounds***

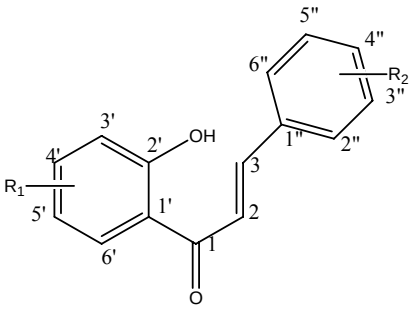

Figure 2: Structure of chalcone derivatives

Comp no	R_1	R_2	molecular formula	mp (ºC)	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

Table 1: ***Physicochemical properties of chalcone derivatives***

Compounds 1a-1j gave positive test for chalcone and positive ferric chloride test.

1a. ^{1}H-NMR (δ/ppm in $CDCl_3$): 5.0 (s, 1H, 2’ -OH), 7.14 (dd, J= 7.9, 1.8Hz, 1H, 4” -H), 7.21 (d, J 7.9 Hz, 2H, 3” , 5 –H”), 7.30 (d, J = 7.9Hz, 2H, 2” , 6” -H), 7.56 (s, 1H, –CH= CH–), 7.64 (m, J 8.3 Hz, 4H, Ar-H), 7.90 (s, 1H, –CH=CH–). IR (KBr/cm^{-1}): 3480(–OH), 1748—1716 (–CO), 1670 (–CH=CH–), 1616, 1558 (aromatic), 754, 697 (monosubstituted benzene).

1b ^{1}H-NMR (δ/ppm in $CDCl_3$): 5.0 (s, 1H, 2’ -OH), 5.1 (s, 1H, 4” -OH), 6.68 (d, J=7.9Hz, 2H, 3” , 5” -H), 7.13 (d, J=8.0Hz, 2H, 2” , 6” -H), 7.64—6.92 (m, J=8.3 Hz, 4H, Ar-H), 7.56 (s, 1H, –CH=CH–), 7.90 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3480, 3345 (–OH),1771, 1732 (–CO), 1682 (–CH=CH–), 1603, 1575 (aromatic), 834 (p-disubstituted benzene).

1c ^{1}H-NMR (δ/ppm in $CDCl_3$): 3.73 (s, 3H, 4” -OCH_3), 5.0 (s, 1H, 2’ -OH), 6.72 (d, J=7.9Hz, 2H, 3” , 5” -H), 7.19 (d, J=7.9Hz, 2H, 2” ,6” -H), 7.56 (s, 1H, –CH=CH–), 7.64—6.92 (m, J=8.1 Hz, 4H, Ar-H), 7.90 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3480, 3446 (–OH), 1748, 1716 (–CO), 1670 (–CH=CH–), 1605, 1575 (aromatic), 834 (p-disubstituted benzene).

1d ^{1}H-NMR (δ/ppm in $CDCl_3$): 2.8 (s, 6H, 4” -NMe_2), 5.0 (s, 1H, 2’ -OH), 6.54 (d, J=7.9 Hz, 2H, 3” , 5” -H), 7.12 (d, J=8.0 Hz, 2H, 2” , 6” - H), 7.56 (s, 1H, –CH=CH–), 7.64—6.92 (m, J=7.9 Hz, 4H, Ar-H), 7.90 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3480, 3446 (–OH),1748, 1716 (–CO), 1670 (–CH=CH–), 1621, 1558, 1521 (aromatic), 1312 (C–N stretching in Ar amines), 835 (p-disubstituted ben-zene).

1e ^{1}H-NMR (δ/ppm in $CDCl_3$): 5.0 (s, 3H, 2’ , 4’ , 6” -OH), 6.68 (d, J=7.9Hz, 2H, 3” , 5” -H), 7.13 (d, J=7.9Hz, 2H, 2” , 4” -H), 7.39 (s, 1H, –CH=CH–), 7.47—6.39 (m, J=8.2 Hz, 3H, Ar-H), 8.17 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3841 (–OH), 1732, 1698 (–CO), 1670 (–CH=CH–), 1616, 1558 (aromatic), 727, 652 (monosubstituted benzene).

1f ^{1}H-NMR (δ/ppm in $CDCl_3$): 2.85 (s, 6H, 4" -NMe_2), 5.0 (s, 2H, 2' , 4' -OH), 6.54 (d, J=7.9Hz, 2H, 3" , 5" -H), 7.12 (d, J=7.9Hz, 2H, 2" , 6" -H), 7.56 (s, 1H, –CH=CH–), 7.47—6.39 (m, J 8.1 Hz, 3H, Ar-H), 7.90 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3480 (–OH), 1748, 1697 (–CO), 1670 (–CH=CH–), 1616, 1540 (aromatic), 1316 (C–N stretching in Ar. amines), 824 (p-disubstituted benzene).

1g ^{1}H-NMR (δ/ppm in $CDCl_3$): 5.0 (s, 2H, 2' , 6" -OH), 7.11—6.75 (m, J=8.2 Hz, 4H, Ar-H), 7.14 (dd, J=7.9, 1.8Hz, 1H, 4" -H), 7.21 (d, J=7.9Hz, 2H, 3" , 5" -H), 7.30 (s, 1H, 2" -H), 7.56 (s, 1H, –CH=CH–), 7.90 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3391, 3209 (–OH), 1748, 1698 (–CO), 1653 (–CH=CH–), 1623, 1576 (aromatic), 728, 697 (monosubstituted benzene)s.

1h ^{1}H-NMR (δ/ppm in $CDCl_3$): 5.0 (s, 3H, 2' , 5' , 6" -OH), 6.68 (d, J=7.9Hz, 1H, 3' -H), 6.77 (dd, J=7.9, 1.8Hz, 1H, 6' -H), 6.97 (dd, J=7.9, 1.8Hz, 1H, 4' -H), 7.11—6.75 (m, J 8.3 Hz, 4H, Ar-H), 7.39 (s, 1H, –CH=CH–), 8.17 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3446 (–OH), 1748, 1698 (–CO), 1670, 1652 (–CH=CH–), 1616, 1540 (aromatic), 714, 673 (monosubstituted benzene).

1i ^{1}H-NMR (δ/ppm in $CDCl_3$): 5.0 (s, 3H, 2' , 5' , 4" -OH), 6.68 (d, J=7.9Hz, 2H, 3" , 5" -H), 7.11—6.75 (m, J=8.3 Hz, 3H, Ar-H), 7.13 (d, J=7.9Hz, 2H, 2" , 6" -H), 7.56 (s, 1H, –CH=CH–), 7.90 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3244 (–OH), 1732, 1698 (–CO), 1683 (–CH=CH–), 1646, 1557 (aromatic), 834 (p-disubstituted benzene).

1j ^{1}H-NMR (δ/ppm in $CDCl_3$): 3.73 (s, 3H, 4" -OCH_3), 5.0 (s, 2H, 2' , 5' -OH), 6.72 (d, J =7.9 Hz, 2H, 3" , 5" -H), 7.11—6.75 (m, J=8.3 Hz, 3H, Ar-H), 7.19 (d, J=7.9Hz, 2H, 2" , 6" -H), 7.56 (s, 1H, –CH=CH–), 7.90 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3244 (–OH), 1732, 1716 (–CO), 1683 (–CH=CH–), 1577, 1540 (aromatic), 834 (p-disubstituted benzene).

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 (**2).** IR (KBr/cm^{-1}) 3451, 1666, 844, 1H-NMR (δ /ppm in $CDCl_3$): 2.14 (s, 3H, CH_3), 7.17-7.63 (m, *J*= 8.2 Hz, 3H, Ar-H) , 8.35 (s, 1H, ArNH, D_2O exchangeable), 9.47 (s, 2H, $CONH_2$, D_2O exchangeable).

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. 1H-NMR (δ /ppm in $CDCl_3$): 2.15 (s, 3H, CH_3), 5.46 (s, 2H, NH_2, D_2O exchangeable), 7.12-7.64 (m, *J*= 8.3 Hz, 4H, Ar-H) , 8.34 (s, 1H, ArNH, D_2O exchangeable), 9.42 (bs, 1H, $NHNH_2$, D_2O exchangeable); IR (KBr/cm^{-1}) 3250, 3038, 2854, 1718, 1620-1555, 1278, 690.

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. 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 3) and physicochemical properties of the synthesized title compounds are given in table 2.

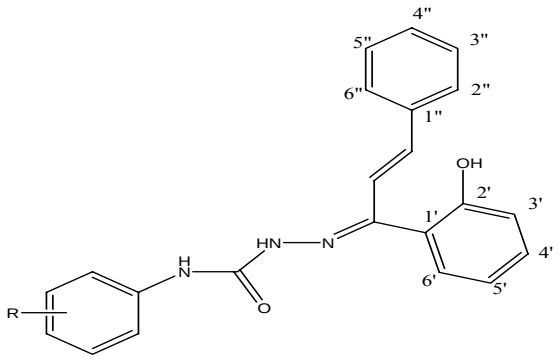

Figure 3: Structure of synthesized title compounds

Comp no.	R	R_1	R_2	Yield (%)	mp (°C)	Rf value
4	2-CH_3	H	H	57	150	0.78
5	2-CH_3	H	4”-OH	66	145	0.71
6	2-CH_3	H	4”-OCH_3	65	135	0.65
7	2-CH_3	H	4”-$N(CH_3)_2$	58	148	0.57
8	2-CH_3	4-OH	6”-OH	57	142	0.60
9	2-CH_3	4-OH	4”-$N(CH_3)_2$	50	160	0.67
10	2-CH_3	H	6”-OH	63	140	0.55
11	2-CH_3	5-OH	6”-OH	61	135	0.63
12	2-CH_3	5-OH	4”-OH	56	120	0.69
13	2-CH_3	5-OH	4”-OCH_3	57	126	0.51
14	4-CH_3	H	H	52	206	0.53
15	4-CH_3	H	4”-OH	65	188	0.63
16	4-CH_3	H	4”-OCH_3	63	204	0.70
17	4-CH_3	H	4”-$N(CH_3)_2$	64	195	0.62
18	4-CH_3	4-OH	6”-OH	55	178	0.58
19	4-CH_3	4-OH	4”-$N(CH_3)_2$	56	185	0.66
20	4-CH_3	H	6”-OH	54	180	0.69
21	4-CH_3	5-OH	6”-OH	67	183	0.54
22	4-CH_3	5-OH	4”-OH	50	165	0.59
23	4-CH_3	5-OH	4”-OCH_3	56	172	0.77

Table 2: ***Physicochemical properties of synthesized title compounds***
(Mobile phase: chloroform: methanol 9:1)

1-[1-(2-hydroxyphenyl)-3-phenylallylidene]-4-(2-methylphenyl)semicarbazide **(4):**
1H-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).

1-[1-(2-hydroxyphenyl)-3-(4-hydroxyphenyl)allylidene]-4-(2-methylphenyl) semicarbazide **(5)**
1H-NMR (δ/ppm in $CDCl_3$): 2.18 (s, 3H, Ar-CH_3), 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, 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).

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

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

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

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

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

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).

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).

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).

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_2O exchangeable), 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).

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).

Pharmacology

Anti-inflammatory

Animals were divided into control, standard and different test groups comprising of five animals in each group. They were fasted overnight with free access to water before experiment. The anti-inflammatory activity was determined *in vivo* using the carrageenan-induced rat paw edema test [10, 15]. In all groups, acute inflammation was produced by sub-planter injection of 0.1ml of freshly prepared 1% suspension of carrageenan (Sigma-Aldrich, Dorset, UK) in the right hind paw of the rats 1 h after i.p. administration of the compounds and paw volume was measured plethysmometrically at 0 hr and 3hr. The test compounds (30mg/kg) was administered i.p. in DMSO, standard group was treated with diclofenac (50mg/kg) i.p. 1 hrs before by the injection and control group received only DMSO. Anti-inflammatory activity was expressed as percent of inhibition of the edema when compared with the control group. Mean difference in paw volume was measured statistically by student t test (Dunnett). Mean defference in paw volume was measured and percentage inhibition was calculated by using formula

$$\% \text{ inhibition of edema} = (Vc\text{-}Vt / Vc) \times 100,$$

where Vt and Vc are the mean paw volume of test group and control group, respectively.

Results and Discussion

The anti-inflammatory activity of the synthesized compounds is summarized in Table 3. Comparison of the anti-inflammatory activity of all tested compounds revealed that compound 11 was the most active compound in the chalconesemicarbazone series. As can be seen from Table 3, hydroxyl substituted chalconesemicarbazones were potent anti-inflammatory agents. Among the synthesized compounds, compound 11, 12, 20 and 21 showed the better activity in comparison to diclofenac sodium as the reference drug. In

reference to the methyl substitution, the substitution at position 2 was more favorable than the 4 position. But in the case of substitution on phenyl of aldehydic and acetophenic group of chalcone moiety, the hydroxyl substitution favors the increased biological activity, may due to increased hydrogen bonding. The compound 11 was more active in comparison to the reference drug. The unsubstituted compound (4), shown very less activity, may be due to improper attachment with the binding site.

Table 3: ***Anti-inflammatory activity of title compounds***

Compound	Dose(mg/kg)	Thickness variation(mm)	Inhibition (%)
Control	----	0.950±.122	----
Diclofenac Sodium	50	0.160±.010***	83.15
4	30	0.545±.095**	42.63
5	30	0.240±.023**	74.73
6	30	0.397±.021**	58.2
7	30	0.485±.095**	48.94
8	30	0.158±.023**	83.36
9	30	0.427±.021**	55.05
10	30	0.375±.095**	60.52
11	30	0.120±.023**	87.36
12	30	0.153±.021**	83.89
13	30	0.345±.095**	63.68
14	30	0.576±.023**	39.36
15	30	0.427±.021**	55.05
16	30	0.405±.095**	57.36
17	30	0.496±.023**	47.78
18	30	0.187±.021**	80.31
19	30	0.445±.095**	53.15
20	30	0.146±.023**	84.63
21	30	0.153±.024**	83.89
22	30	0.355±.095**	62.63
23	30	0.340±.023**	64.21

a) Number of animals in each group n = 4.

b) Thickness variation is the difference between the thickness of the carrageenan-treated paw and the saline-treated paw.

c) Percentage of inhibition obtained by comparison with the standard drug. * and ** differed from control group $P < 0.05$ and $P < 0.01$, respectively.

In summary, most of the synthesized compounds were potential lead for an anti-inflammatory analgesic activity. On the bases of observed results, it may be concluded that the substitution favours the activity, but the bulkier substitution may also disfavors the activity, may be due to the improper attachment with binding site. The hydroxyl substitution increases the activity of the compounds, may be due to increased hydrogen bonding with the binding site. 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.

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