SYNTHESIS AND EVALUATION OF ANALGESIC ACTIVITY OF CHALCONESEMICARBAZONES

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Summary

A series of chalconesemicarbazones was synthesized and evaluated for their analgesic activities by hot plate and formalin induced paw licking animal model. Most of the compounds were found to be more or comparable potent than the reference standard drug in the both animal models. Based on the results of analgesic study, 1-[1-(2-hydroxyphenyl)-3-(2-hydroxyphenyl)allylidene]-4-(2-methylphenyl) semicarbazide **(**11) was the most active compound.

Keywords: Chalcones, Analgesic activity, Semicarbazones, Hot plate

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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]. There are several reports about the synthesis and pharmacological evaluation of new bioactive N-aroylarylhydrazones acting at the AA cascade enzyme level and chalcones are also having analgesic activity [2-9]. As a part of ongoing research program to find novel analgesic compounds, herein, we have fused these both active moiety and design a scheme for synthesizing these [10-12]. The analgesic (anti-nociceptive) of synthesized compounds was performed using hot plate and formalin induced paw licking methods.

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 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.

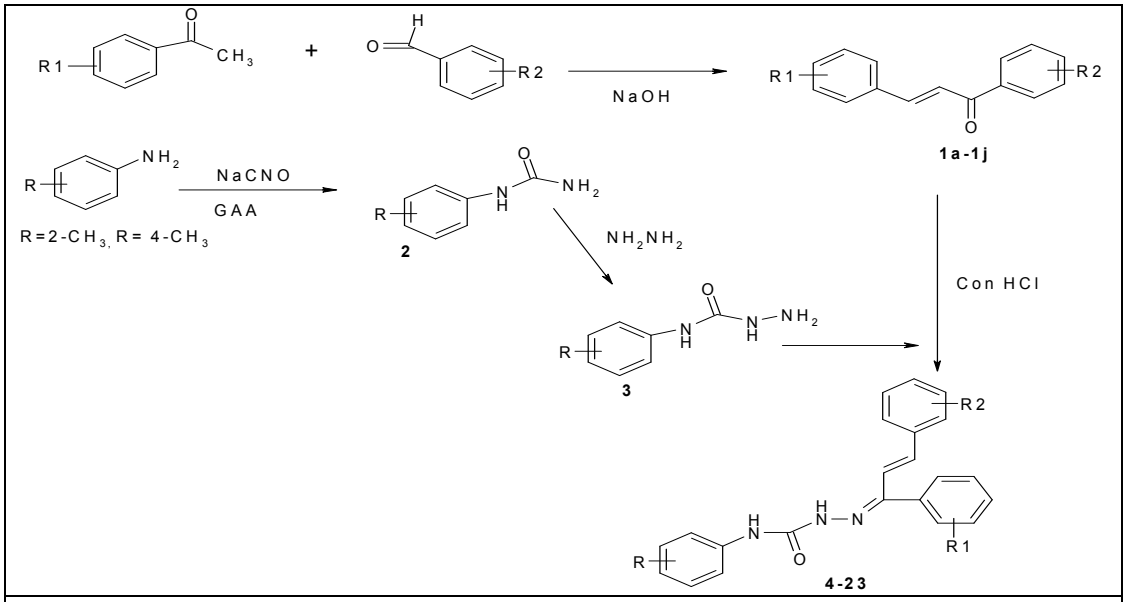

Figure 1: synthetic scheme for synthesizing the title compounds

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.

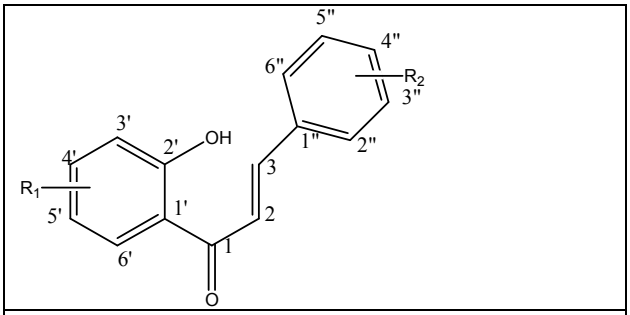

Figure 2: Structure of chalcone derivatives

Table 1: ***Physicochemical properties 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

Compounds 1a-1j gave positive test for chalcone.

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.

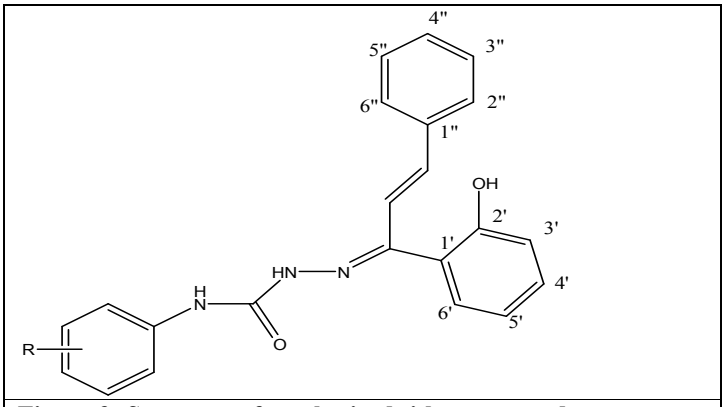

Figure 3: Structure of synthesized title compounds

Table 2: ***Physicochemical properties of synthesized title compounds***

Comp no.	R	R_1	R_2	Yield (%)	mp (ºC)	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

(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

Animals

Male wistar rats (weighing 180 ± 10 g) were used for the study. The rats were bred and housed in the School of Pharmaceutical Sciences, Animal house, Jaipur National University, Jaipur, Rajasthan, India. Animals were housed in groups of six in a room at a constant temperature of 25±2°C with 55±10% relative humidity.The animal house was well ventilated and the normal day light cycle was maintained. Animals were divided into groups comprising of six rats each. Animals were fed with standard rat pellet and water *ad libitum.*

Analgesic activity

The synthesized Chalconesemicarbazones were screened for analgesic activities using the hot plate test and the formalin induced paw licking test.

In the Hot plate (Eddy's Hot Plate; Techno, India) test animals were orally administered 30 mg/Kg of the Compound, saline (control) or 100 mg/Kg Aspirin (Reference drug). The animals were each placed on a hot plate (maintained at 55 ± 1^{o}C) after 60 min of the administration of the Compound, drug or Saline and the time (Reaction time) it takes each of the animals to jump off the hot plate was noted and cut off period of 15 sec.The mean of the responses for the animals (6 per group) administered each compound was compared with the control group [13].
In the Formalin test, the paws of the animals were injected with 100 µL of 3% formalin after one hour of the oral administration of Compound (30 mg/Kg), saline or Aspirin (!00mg/kg. The licking time in the first phase (0 - 5 min post formalin injection) and in the second phase (20 – 30 min post formalin injection) was noted and the mean for each group was determined and compared with the control group [14-15].

Results and discussion

The Analgesic activity of the synthesized compounds by hot plate method is summarized in Table 3 & Figure 4.

Table 3: Effects of the synthesized chalconesemicarbazones on hot plate test in rats

Compound	Dose mg/kg	Reaction Time in Seconds (Mean ± SD)[a]	% increase in pain Threshold
Control	--	3.82 ± 0.159	--
Aspirin	100	9.45 ± 0.245*	147.38
4	30	5.95 ± 0.133*	55.76
5	30	9.97 ± 0.161*	160.99
6	30	9.09±0.174*	137.96
7	30	6.93±0.157*	81.41
8	30	7.84±0.235*	105.24
9	30	7.94± 0.211*	107.85
10	30	8.03±0.15*	110.21
11	30	10.56±0.175*	176.44
12	30	10.14±0.213*	165.45
13	30	9.18±0.231*	140.31
14	30	5.55±0.214*	45.29
15	30	9.94±0.213*	160.21
16	30	8.05±0.217*	110.73
17	30	6.89±0.227*	80.37
18	30	8.98±0.21*	135.08
19	30	7.47±0.198*	95.55
20	30	8.05±0.204*	110.73
21	30	10.05±0.212*	163.09
22	30	8.47±0.189*	121.73
23	30	9.11±0.229*	138.48

[a]Each value is the mean □□S.D. for 6 rats.
*P < 0.05 compared with control; One way Anowa test.

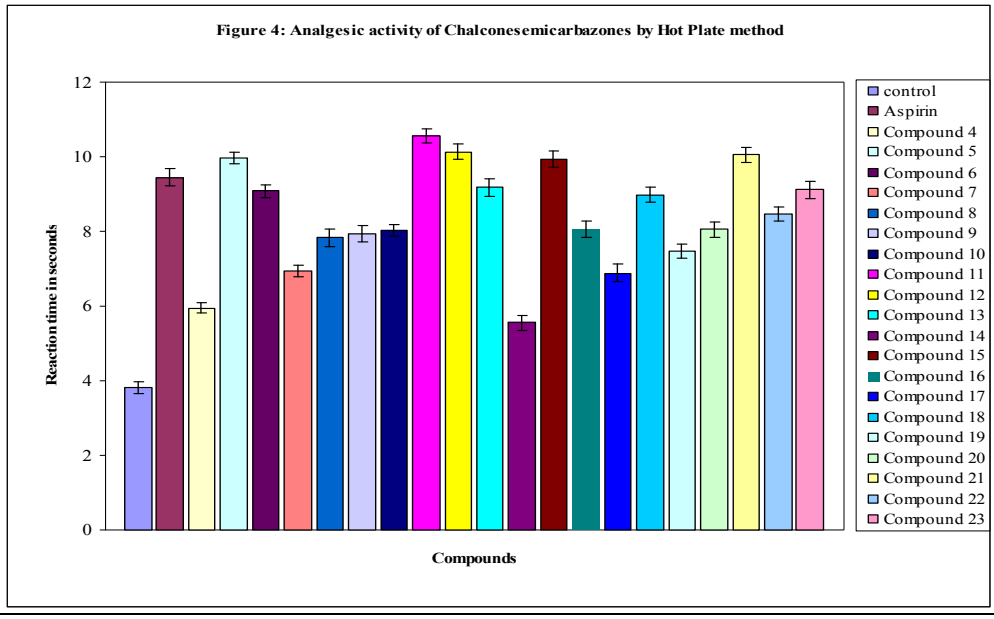

Figure 4: Effects of the synthesized chalconesemicarbazones on hot plate test in rats

The Analgesic activity of the synthesized compounds by Formalin induced paw licking method is summarized in Table 4 & Figure 5.

Table 4: Effects of the synthesized chalconesemicarbazones on Formalin-induced paw-licking in rats.

Compound	Dose(mg/kg) Orally	Formalin induced paw licking			
		Early phase (seconds)[a]	Late Phase (Seconds)[a]	% inhibition early phase	% inhibition late phase
Control	-----	122.5±10.37*	101.67±6.95*	------	-----
Aspirin	50	31.17±5.46*	20.33±4.55*	74.56	80
4	30	90.33±13.06*	79.83±9.99*	26.26	21.48
5	30	38.17±5.31*	29.67±7.26*	68.84	70.82
6	30	44.5±2.07*	32.33±5.61*	63.67	68.2
7	30	76.67±7.17*	62.83±9.97*	37.41	38.2
8	30	64.33±5.54*	48.5±10.67*	47.49	52.3
9	30	67.5±6.66*	53.5±6.75*	44.9	47.38
10	30	55.17±5.98*	44.83±6.18*	54.96	55.91
11	30	25.83±6.014*	19.17±6.34*	78.91	81.15
12	30	29.5±3.83*	21.33±5.47*	75.92	79.02
13	30	39.33±5.39*	37.5±6.19*	67.89	63.12
14	30	90.83±5.98*	75.17±12.37*	25.85	26.07
15	30	33.33±5.16*	30.33±7.42*	72.79	70.17
16	30	50.67±5.61*	45.5±6.28*	58.64	55.25
17	30	82.33±6.65*	68±11.92*	32.79	33.12
18	30	39.83±5.19*	33.1±8.99*	67.49	67.38
19	30	70.33±5.47*	48.67±8.73*	42.59	52.13
20	30	48.67±8.87*	44.83±4.07*	60.27	55.91
21	30	31.67±4.32*	27.83±8.79*	74.15	72.63
22	30	43.17±4.99*	36.67±4.41*	64.76	63.93
23	30	40.67±6.06*	34.17±6.94*	66.8	66.39

[a]Each value is the mean □□S.D. for 6 rats. *P < 0.05 compared with control; One way Anowa test.

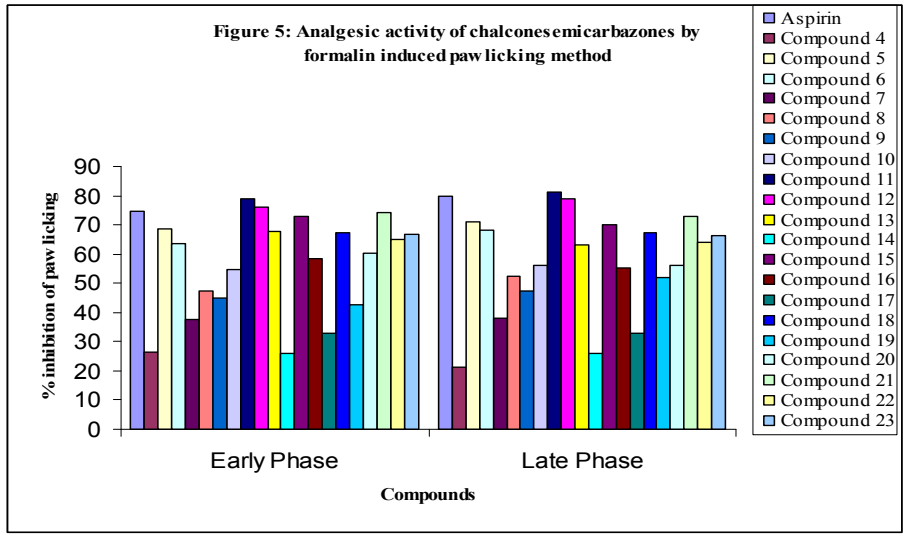

Figure 5: Effects of the synthesized chalconesemicarbazones on formalin induced paw licking test in rats

Comparison of the analgesic 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 & 4, hydroxyl substituted chalconesemicarbazones were potent analgesic agents. Among the synthesized compounds, compound 5, 11, 12, 13, 18, 21 and 23 showed the better activity in comparison to Aspirin 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.

In summary, most of the synthesized compounds were potential lead for 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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