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Research Article

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

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

Keywords: Chalcones, Analgesic activity, Semicarbazones

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-nociceptive activity. As a part of our ongoing research program [11–14] to find novel analgesic compounds, herein, we have fused these both active moiety and design a scheme for synthesizing these. The analgesic (anti-nociceptive) 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.

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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.

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-

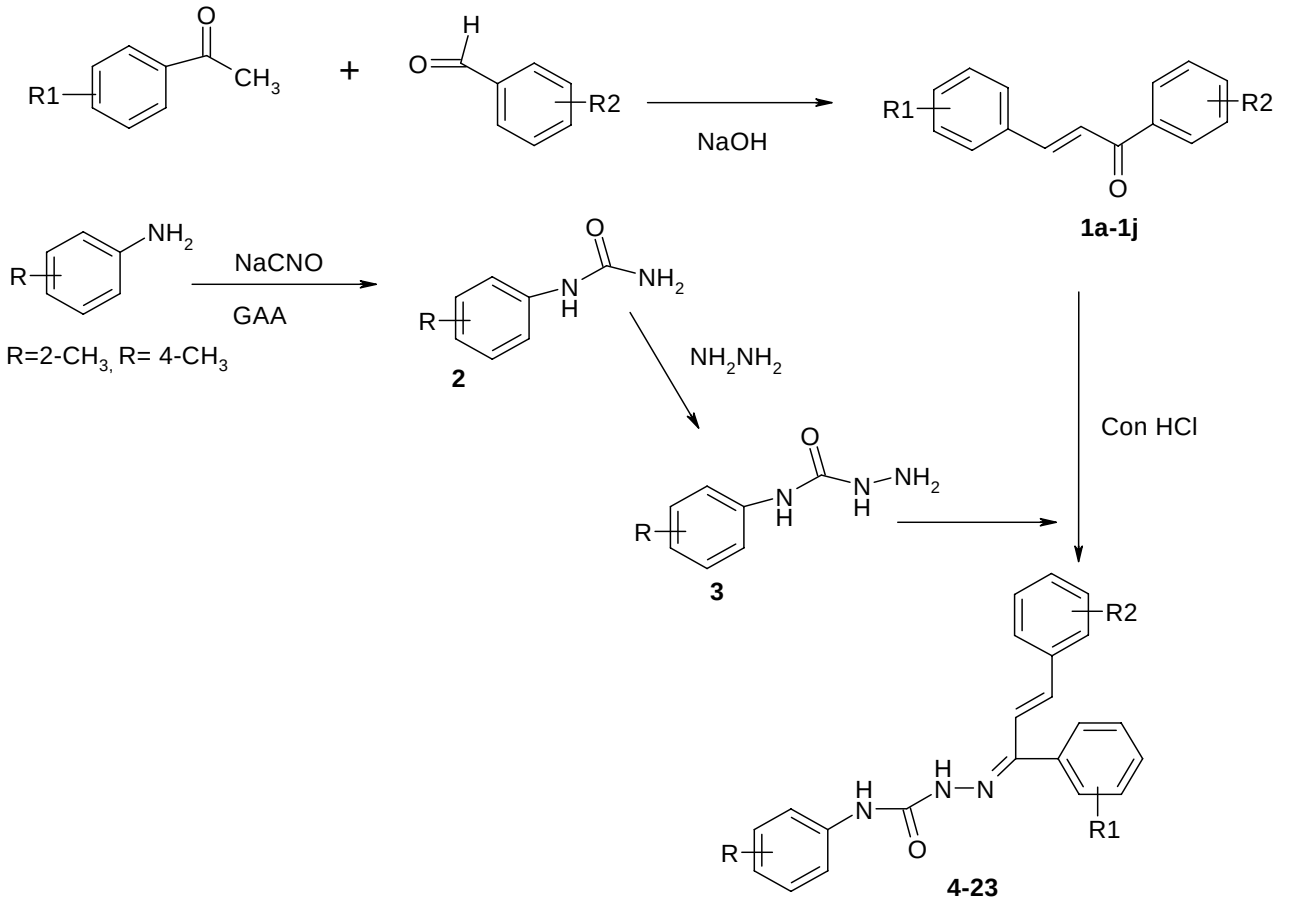

Figure 1: ***synthetic scheme for synthesizing the title compounds***

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).

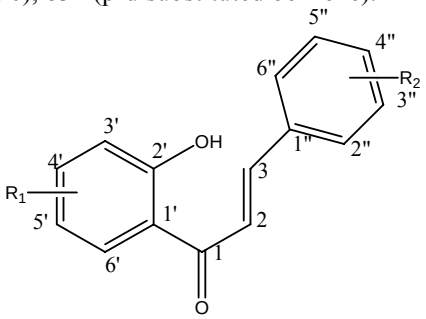

Figure 2: Structure of chalcone derivatives

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).

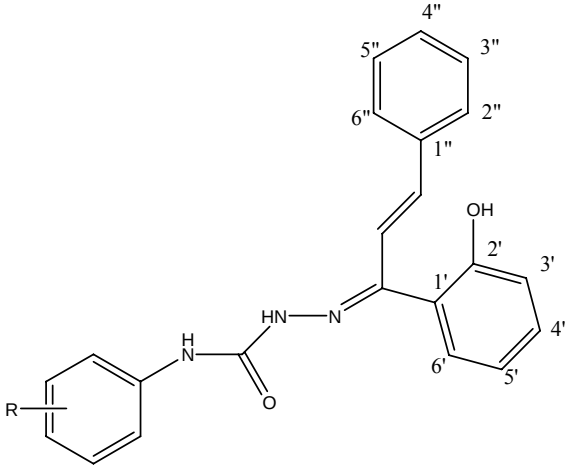

Figure 3: Structure of synthesized title compounds

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,

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

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).

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)

Table 3: ***Antinociceptive activity of title compounds***

Compound	Dose(mg/kg)	Number of writhings (mean ± SEM)	Activity (%)
Control	--	83 ± 6.72	----
Aspirin	50	24.75 ± 2.66**	70.18
4	30	42.5 ± 5.12*	48.79
5	30	17.12 ± 4.41**	79.37
6	30	25 ± 5.58**	69.87
7	30	40 ± 4.46	51.80
8	30	28 ± 5.21*	66.26
9	30	29.5 ± 7.59*	64.45
10	30	27 ± 7.26*	67.46
11	30	12.5 ± 4.48**	84.93
12	30	14 ± 3.10**	83.13
13	30	21.05 ± 3.24**	74.63
14	30	45.75 ± 5.80	44.87
15	30	18.25 ± 7.05**	78.01
16	30	26.50 ± 4.53*	68.07
17	30	36 ± 10.52	56.62
18	30	24.75 ± 3.81*	70.18
19	30	31.75 ± 6.30	61.74
20	30	28.5 ± 3.2*	65.66
21	30	14.25±11.27**	82.83
22	30	26.5 ± 2.33*	68.07
23	30	22.0 l ±3.52**	73.48

a) Number of animals in each group n = 6.
b) Percentage if inhibition obtained by comparison with vehicle control group.
c) Analgesic activity relative to aspirin. * and ** differed from control group P < 0.05 and P < 0.01, respectively.

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.

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)**

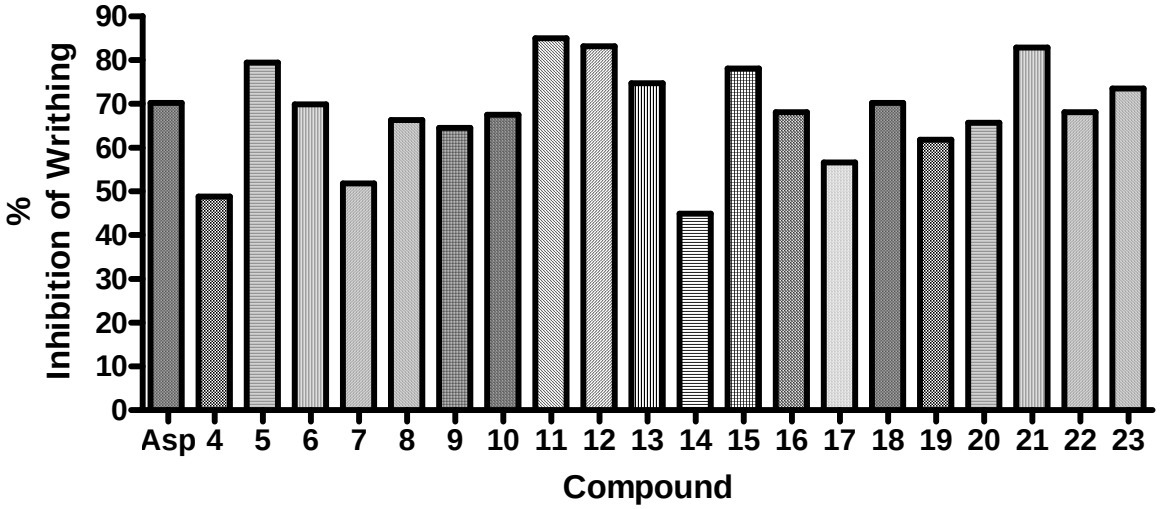

Figure 4: Compounds vs % inhibition of Writhing ; Asp: Aspirin

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

Analgesic activity

The peripheral analgesic activity was evaluated using acetic acid-induced writhing test in mice. In this method, Swiss albino mice of either sex weighing between 25-30 gm were randomly distributed in six groups of six mice each. The first group served as control and the animals of that group were administered 1% v/v acetic acid (1 ml/100 g) intraperitoneally. The onset and the number of writhing were recorded for a period of 10 min for each animal of the group. The second group of animals administered aspirin (50 mg/kg, i.p.) and 30 min later, acetic acid was administered to the animals of that group. The onset and the frequency of writhing response were observed. The animals of remaining groups were treated with drug in DMSO 30 mg/kg and the acetic acid-induced writhing were recorded as described for group 1 and 2. Mean difference was statistically measured by student t test (Dunnett). Percent protection against acetic acid induced writhing was calculated using the formula

% protection = (Nc - Nt / Nc) x 100

where Nt and Nc are the mean values of number of writhing in the test and control group, respectively.

RESULTS AND DISCUSSION

The anti-nociceptive activity of the synthesized compounds is summarized in Table 3 & Figure 4. Comparison of the anti- nociceptive 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-nociceptive 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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REFERENCES

1. D. A. McCormick, D. Contreras, 2001. Annu. Rev. Physiol. 63: 815- 846.
2. O.J. McNamara, Pharmacological Basis of Therapeutics; Hardman, J. G., Limbird, L.E., Gilman, A. G., Eds.; McGraw-Hill: New York, 521-548 (2001).
3. K. J. J. Meador, 2003. Clin. Psychiatry. 64 (Suppl. 8): 30-34.
4. Z. Lin, P. K. Kadaba, 1997. Med. Res. Rev. 17: 537-572.
5. P. Yogeeswari, J. Raghavendran, R. Thirumurugan, A.. Saxena and D. Sriram, 2004. Curr. Drug Targets., 5: 553-568.
6. J. Dimmock, D. Elias, M. Beazely, M. N. Kandepu, 1999. Curr. Med. Chem. 6: 1125.
7. Ni L. , C. Meng, J. Sikorski, 2004. Expert Opin. Ther., Pat. 14: 1669.
8. S. S. Mokle, M. Sayeed, Kothawar and Chopde, 2004, Int. J. Chem. Sci., 2, 1: 96.
9. H. K. Hsieh, L. T. Tsao and J. P. Wang, 2000. J. Pharm. Pharmacol., 52: 163.
10. G. S. Viana, M. A. Bandeira, F. Matos, 2003. J. Phytomedicine. , 10: 189.
11. L. M. Zhao, H. S. Jin, L. P. Sun, H. R. Piao and Z. S. Quan, 2005 Bioorg. Med. Chem. Lett., 15: 5027.
12. J. Dimmock, K. K. Sidhu, R. S. Thayer, P. Mack, M. J. Dutty, R. S. Reid and J. Quail, 1993. J. Med. Chem. 36: 2243–2252.
13. S. N. Pandeya, I. Ponnilavarasan, A. Pandey, R. Lakhan and J. P. Stables, 1999. Pharmazie, 54: 923–925.
14. Ardeshir Rineh, N. Mahmoodi, M. Abdollahi, A. Foroumadi, M. Sorkhi and A. Shafiee, 2007. Arch. Pharm. Chem. Life Sci. 340: 409 – 415.