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Evaluation of Anti-Phlogistic Activity of Synthesized Chalconesemicarbazone derivatives

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ABSTRACT

In present study, a series of chalconesemicarbazones was synthesized and evaluated for their anti-phlogistic (anti-inflammatory) activities. Most of the compounds were found to be potent anti-phlogistic agent in formalin induced paw edema and cotton pellet induced granuloma in rats. Based on the results of an anti-inflammatory study, 1-[1-(2-hydroxyphenyl)-3-(2-hydroxyphenyl)allylidene]-4-(2-methylphenyl) semicarbazide (CS-6) was the most active compound. It was found that hydroxyl substituted chalconesemicarbazones were potent anti-phlogistic agents and unsubstituted compound 1-[1-(2-hydroxyphenyl)-3-phenylallylidene]-4-(2-methylphenyl)semicarbazide (CS-1) and 1-[1-(2-hydroxyphenyl)-3-phenylallylidene]-4-(4-methylphenyl)semicarbazide (CS-2) showed very less activity.

Keywords: Chalcones, Anti-inflammatory activity, Semicarbazones, anti-phlogistic agent

INTRODUCTION

Antiphlogistic agents are also called anti-inflammatory agents which minimize the vasodilation/vascular permeability/inflammation induced by phlogistic agents. Non steroidal anti-inflammatory drugs (NSAID's) like aspirin are widely used in the treatment of pain and inflammation. NSAID's minimizes the pain and swelling 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 Anti-phlogistic (anti-inflammatory) activity of synthesized compounds was performed using formalin induced paw edema and cotton pellet induced granuloma in rats.

EXPERIMENTAL SECTION

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.

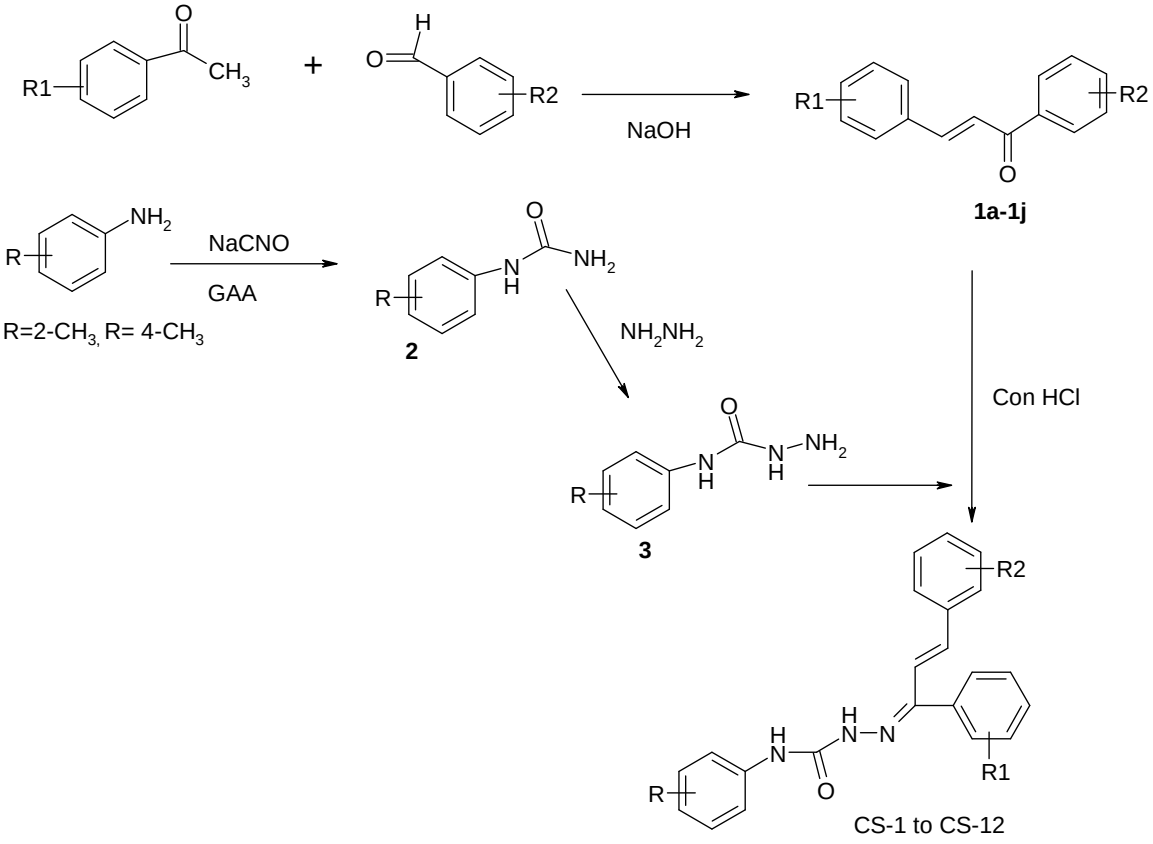

Figure 1: synthetic scheme for synthesizing the title compounds

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.

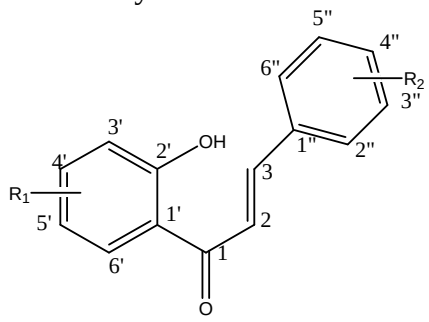

Figure 2: Structure of chalcone derivatives

The structure (figure 2) and 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 (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

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.

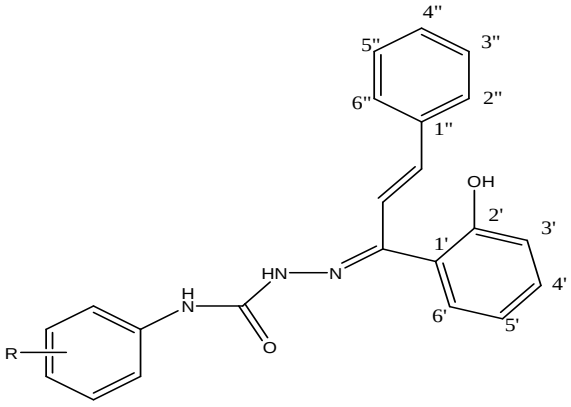

Figure 3: Structure of synthesized title compounds

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

Table 2: *Physicochemical properties of synthesized title compounds*

Comp no.	R	R_1	R_2	Yield(%)	mp(ºC)	Rf value
CS-1	2-CH_3	H	H	57	150	0.78
CS-2	4-CH_3	H	H	52	206	0.53
CS-3	4-CH_3	H	4”-OCH_3	63	204	0.70
CS-4	4-CH_3	4-OH	6”-OH	55	178	0.58
CS-5	4-CH_3	5-OH	4”-OCH_3	56	172	0.77
CS-6	2-CH_3	5-OH	6”-OH	61	135	0.63
CS-7	4-CH_3	H	4”-OH	65	188	0.63
CS-8	4-CH_3	4-OH	4”-$N(CH_3)_2$	56	185	0.66
CS-9	4-CH_3	H	4”-$N(CH_3)_2$	64	195	0.62
CS-10	4-CH_3	H	6”-OH	54	180	0.69
CS-11	4-CH_3	5-OH	6”-OH	67	183	0.54
CS-12	4-CH_3	5-OH	4”-OH	50	165	0.59
(Mobile phase: chloroform: methanol 9:1)						

1-[1-(2-hydroxyphenyl)-3-phenylallylidene]-4-(2-methylphenyl)semicarbazide **(CS-1):**
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-phenylallylidene]-4-(4-methylphenyl)semicarbazide **(CS-2):**
^{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-methoxyphenyl)allylidene]-4-(4-methylphenyl) semicarbazide **(CS-3)**

^{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 **(CS-4)**

^{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-(2-methylphenyl) semicarbazide **(CS-6)**

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-hydroxyphenyl)-3-(4-hydroxyphenyl)allylidene]-4-(4-methylphenyl) semicarbazide **(CS-7)**

^{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-(2-hydroxyphenyl)allylidene]-4-(4-methylphenyl) semicarbazide **(CS-10)**

^{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 **(CS-12)**

^{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).

Pharmacological Evaluation

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 four rats (cotton pellet granuloma) or six rats (formalin induced paw edema) each. Animals were fed with standard rat pellet and water *ad libitum.*

Antiphlogistic activity

In the cotton pellet granuloma, 30 mg of cotton pellet were surgically inserted into the groin of animals for 7 days with the administration of Chalconesemicarbazones (30 mg/Kg), Saline or aspirin (100mg/kg) once a day for the 7 days period. On the eighth day animals were sacrificed with an overdose of ether. The cotton pellets with the attached granuloma were dissected out, dried and the weights of the dried granuloma were determined. The mean of the granuloma formed in each animal was determined [13-14].

In the formalin induced paw edema, Just before injection of the test compounds the volume of the paw was measured plethysmographically. Animals were pretreated of either test compounds (30mg/kg, p.o.) or aspirin (100mg/kg, p.o.). The control group received the same volume of the vehicle. Edema was induced after one hour by subplanter injection of 0.05 ml of a 2.5% solution of formalin into the left hind paw. The increase in paw volume was determined 4 h after injection of the phlogistic agent. The percentage anti-inflammatory activity was calculated by the formula: anti-inflammatory activity=(1-dt/dc)/100 where dt is the difference of paw volume in drug treated groups and dc is the difference in paw volume of the control group [15-16].

RESULTS AND DISCUSSION

The anti-phlogistic activity of the synthesized compounds by cotton pellet induced granuloma is summarized in Table 3 and figure 4.

Table 3: Effects of the chalconesemicarbazones on Cotton pellet-induced granuloma in rats

Group	Dose (mg/kg p.o.)	Increase in weight of pellet (mg)[a]	Inhibition (%)
Control	----	0.69±0.022	----
Aspirin	100	0.11±0.01*	23.19
CS-1	30	0.53±0.039*	23.19
CS-2	30	0.58±0.022*	15.94
CS-3	30	0.46±0.022*	33.33
CS-4	30	0.2±0.026*	71.01
CS-5	30	0.33±0.035*	52.17
CS-6	30	0.120±0.014*	82.61
CS-7	30	0.48±0.069*	30.44
CS-8	30	0.45±0.027*	34.78

CS-9	30	0.51± 0.032*	26.09
CS-10	30	0.14±0.018*	79.71
CS-11	30	0.15±0.026*	78.26
CS-12	30	0.36±0.018*	47.83
[a] Each value is the mean±S.D. for 4 rats. *P<0.05 compared with control; One way ANOWA test			

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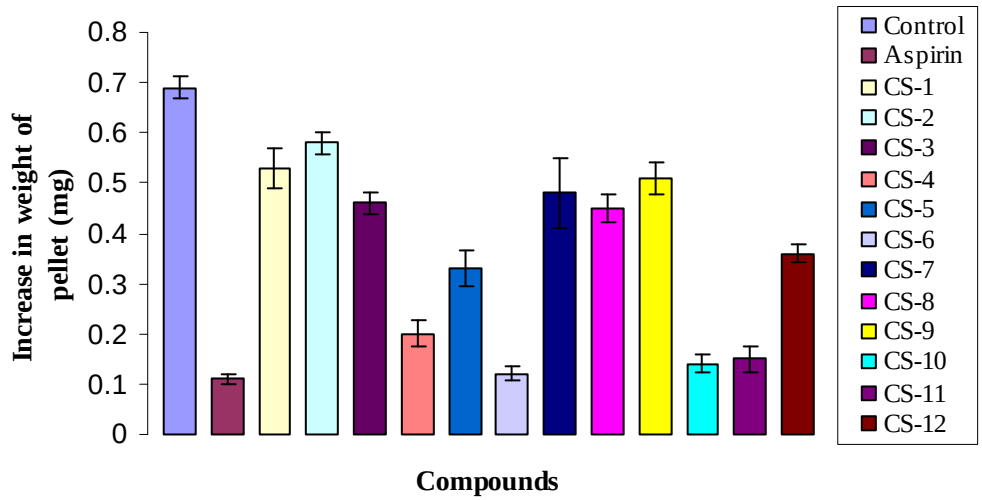
Figure 4. Antiphlogistic activity of chalconesemicarbazones by cotton pellet granuloma method



The anti-phlogistic activity of the synthesized compounds by formalin induced paw edema is summarized in Table 4 and figure 5.

Figure 5. Antiphlogistic activity of chalconesemicarbazones on formalin induced paw edema in rats

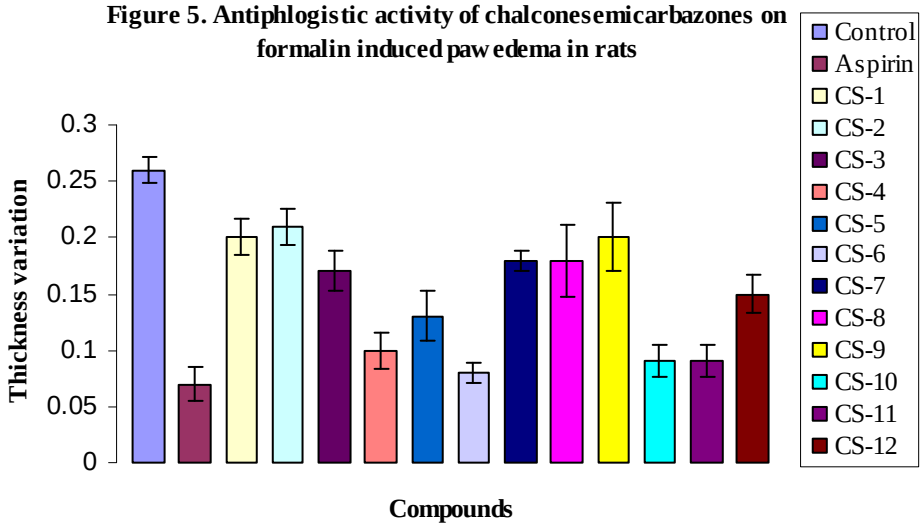


Table 4: Effects of the chalconesemicarbazones on formalin induced paw edema in rats

Group	Dose (mg/kg p.o.)	Thickness variation (mm)[a]	Inhibition (%)
Control	----	0.26±0.012*	----
Aspirin	100	0.07±0.015*	73.08
CS-1	30	0.2±0.016*	23.08
CS-2	30	0.21±0.016*	19.23
CS-3	30	0.17±0.018*	34.62
CS-4	30	0.1±0.016*	61.54
CS-5	30	0.13±0.022*	50
CS-6	30	0.08±0.009*	69.23
CS-7	30	0.18±0.009*	30.77
CS-8	30	0.18±0.032*	30.77
CS-9	30	0.2±0.03*	23.08
CS-10	30	0.09±0.014*	65.38
CS-11	30	0.09±0.014*	65.38
CS-12	30	0.15±0.016*	42.31
[a] Each value is the mean±S.D. for 6 rats. *P<0.05 compared with control; One way ANOWA test.			

Comparison of the antiphlogistic activity of all tested compounds revealed that compound CS-6 was the most active compound in the chalconesemicarbazone series. As can be seen from Table 3 and 4, hydroxyl substituted chalconesemicarbazones were potent anti-inflammatory agents. Among the synthesized compounds, compound **CS-4, CS-6, CS-10 and CS-11** showed the good antiphlogistic activity that was comparable 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 unsubstituted compound (CS-1 and CS-2), showed very less activity, which may be due to improper attachment with the binding site.

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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