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Design, Synthesis, Analgesic and Anti-Inflammatory Activity of Some novel Chalconesemicarbazone derivatives

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Abstract

In the present study we have designed a new pharmacophore 'Chalconesemicarbazone' by pharmacophore hybridization approach of drug design. A series of novel 'chalconylsemicarbazide' derivatives was synthesized. The synthesized compounds were evaluated for their analgesic and anti-inflammatory activities. Most of the compounds were found to be more or comparable potent than the reference standard drugs. Based on the pharmacological screening results 1-(1,5-diphenylpenta-2,4-dienylidene)-4-(2-nitrophenyl) semicarbazide (15) was the most active lead compound.

Keywords: Chalcones, Analgesic activity, 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 [1-3]. Additionally, the 5-lipoxygenase (5-LO) products such as leukotriene B4 (LTB4) also contributes to the hyperalgesia seen during inflammation by decreasing the mechanical and thermal thresholds of C fibers [4]. Leukotrienes, especially LTB4 together with prostaglandins are implicated in the acute ulceration induced by NSAID's [6]. For these reasons, compounds that achieve dual inhibition of the enzymes COX and 5-LO reduce side effects and improved the efficacy in the combat of pain in inflammatory diseases [7]. Some previously synthesized hydrazone analogues were also described as a dual COX/5-LO inhibitor [8,9]. Furthermore, there are several reports about the synthesis and pharmacological evaluation of new bioactive N-aryl-

arylhydrazones acting at the AA cascade enzyme level (Fig. 1) [11, 12, 14]. As a part of research program [15-17] Shafiee etal also find analgesic and anti-inflammatory activity of 4-(2-phenoxyphenyl)semicarbazones [21].

Chalcones belong to the flavonoid family from plant origin and some of them possess antiinflammatory activity. Recently, several natural and synthetic chalcone derivatives were reported to inhibit inducible nitric oxide synthase (iNOS)-catalyzed NO production in cell cultures [22]. Butein, a natural chalcone, has anti-inflammatory activity. One synthetic derivative of butein, 2',4',6'-tris(methoxymethoxy)chalcone (TMMC), has potent anti-inflammatory activity via an HO-1 (haeme-oxygenase) dependent pathway [23].

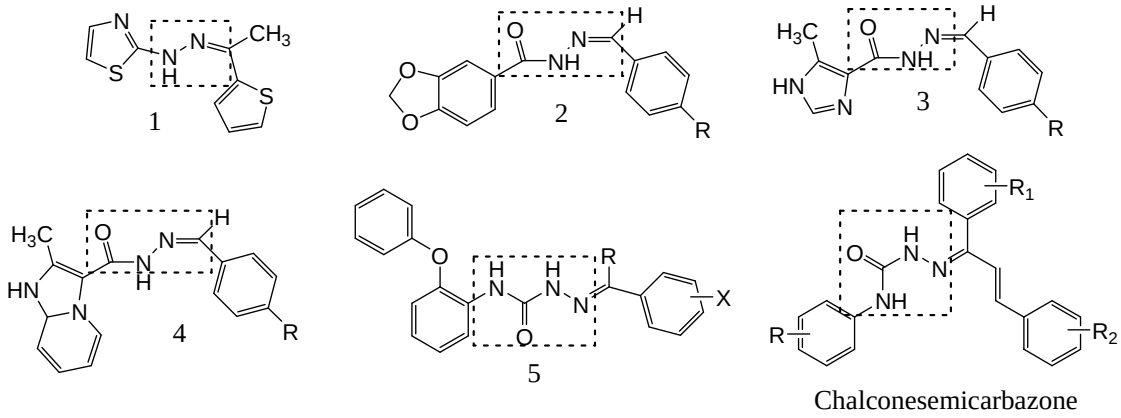

Figure 1: structures of some hydrazones, phenoxy semicarbazones and chalconsemicarbazone

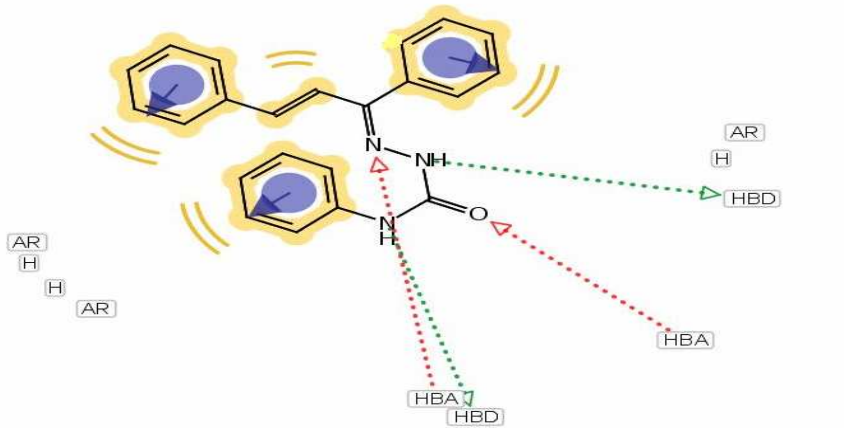

Figure 2: pharmacophore of the designed chalconesemicarbazone

From the literature it have also been concluded that the semicarbazone [21] and chalcone, both the moieties have good anti-inflammatory and analgesic activities. In the present study we have used pharmacophore hybridization technique of drug design and designed a pharmacophore model 'chalconesemicarbazone', which is having hydrogen acceptor site, hydrogen donor site, lipophilic site etc (figure 2), which may help in binding with receptors and plays an important role in pharmacological activities. On these observations, we have designed a synthetic scheme to synthesize this pharmacophore, and also synthesize some lead compounds. We have also done the pharmacological screening as anti-inflammatory and analgesic agents. 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.

MATERIALS AND METHODS

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.

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

Synthesis of 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 $CDCl_3$): 7.17-7.63 (m, 4H, 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 $CDCl_3$): 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.

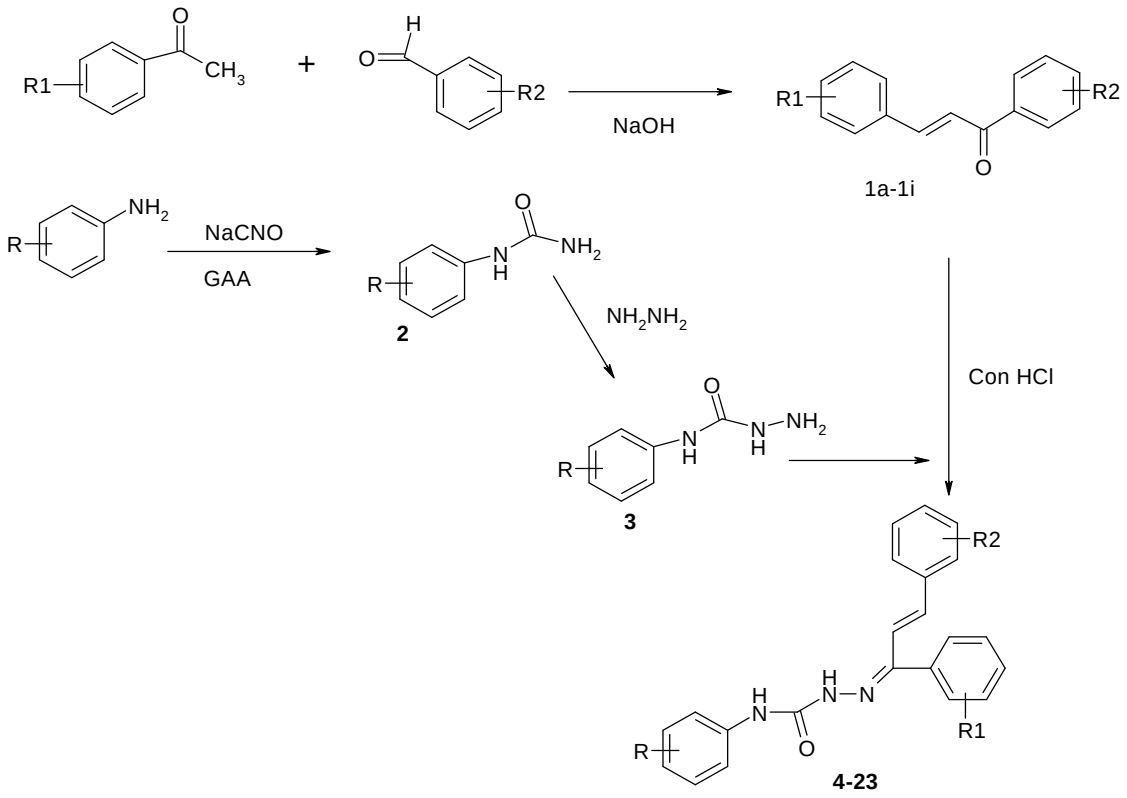

Figure 3: synthetic scheme for synthesizing the title compounds

General method for the synthesis of substituted phenyl chalconesemicarbazone **(4-22)**
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 and physicochemical properties of the synthesized title compounds are given in table 1.

Table 1: Structure and physicochemical properties of synthesized title compounds

Com	R	R_1	R_2	M Formula	yield (%)	Mp (°C)	Rf
4	H	H	H	$C_{22}H_{19}N_3O$	72	55	0.78
5	H	H	p-$(CH_3)_2N$	$C_{24}H_{24}N_4O$	72	74	0.71
6	H	H	p-Cl	$C_{22}H_{18}ClN_3O$	75	93	0.65
7	H	H	Cinnameldehyde	$C_{24}H_{21}N_3O$	75	84	0.57
8	H	p-NH_2	H	$C_{22}H_{20}N_4O$	64	78	0.60
9	H	p-NH_2	p-Cl	$C_{22}H_{19}ClN_4O$	68	158	0.67
10	p-Cl	H	H	$C_{22}H_{18}ClN_3O$	70	197	0.55
11	p-Cl	H	Cinnameldehyde	$C_{24}H_{20}ClN_3O$	72	91	0.63
12	o-Cl	H	p-Cl	$C_{22}H_{17}Cl_2N_3O$	72	93	0.69
13	o-Cl	p-NH_2	p-Cl	$C_{22}H_{18}Cl_2N_4O$	60	152	0.51
14	o-Cl	H	Cinnameldehyde	$C_{24}H_{20}ClN_3O$	64	80	0.53
15	o-NO_2	H	Cinnameldehyde	$C_{24}H_{20}N_4O_3$	64	NA	0.63
16	p-Br	H	H	$C_{22}H_{18}ON_3Br$	70	158-160	0.86[a]
17	p-Br	H	o-OH	$C_{22}H_{18}O_2N_3Br$	74		-
18	p-Br	H	p-$(CH_3)_2NH$	$C_{24}H_{23}ON_4Br$	65	76-80	0.90[a]
19	p-Br	H	p-Cl	$C_{22}H_{17}ON_3BrC$	62	154-156	0.74[a]
20	p-Br	H	Cinnameldehyde	$C_{24}H_{20}ON_3Br$	75	216-220	0.89[a]
21	p-Br	p-OH	H	$C_{22}H_{18}O_2N_3Br$	67	176-180	0.23[a]
22	p-Br	p-OH	p-$(CH_3)_2N$	$C_{24}H_{23}O_2N_4Br$	74	208-210	0.67[a]

(Mobile phase: chloroform: methanol 9:1)

*4-phenyl-1-(1,3-diphenyl allylidene)semicarbazide***(4)**
1H-NMR (δ/ppm in $CDCl_3$): 7.11-7.64 (m, 15H, 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), 3300–3240 (CONH), 1670 (–CH=CH–),1590 (C-N), 1616, 1558 (aromatic), 754, 697 (monosubstituted benzene); MS, m/z 340; Elemental analysis calculated/found (%) C (77.40/77.26), H (5.61/5.48), N (12.31/12.12).

[3-{4-(dimethylamino) phenyl}-1-phenylallylidene]-4-phenyl semicarbazide **(5)**
1H-NMR (δ/ppm in $CDCl_3$): 3.14 (s, 6H, CH_3)7.11-7.64 (m, 14H, Ar-H), 7.76 (s, 1H, –CH=CH–), 7.92 (s, 1H, –CH=CH–), 8.36 (s, 1H, ArNH, D_2O exchangeable), 9.32 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3452 (NH), 3310–3242 (CONH), 1673 (–CH=CH–),1592 (C-N), 1619, 1555 (aromatic), 752, 693 (monosubstituted benzene); MS, m/z 383; Elemental analysis calculated/found (%) C (74.97/74.88), H (6.29/6.18), N (14.57/14.52).

[3-(4-chloro phenyl)-1-phenylallylidene]-4-phenylsemicarbazide **(6)**
1H-NMR (δ/ppm in $CDCl_3$): 7.11-7.64 (m, 14H, Ar-H), 7.65 (s, 1H, –CH=CH–), 7.91 (s, 1H, –CH=CH–), 8.36 (s, 1H, ArNH, D_2O exchangeable), 9.45 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3450 (NH), 3300–3248 (CONH), 1662 (–CH=CH–),1588 (C-N), 1613, 1558

(aromatic), 878 (Cl), 753, 692 (monosubstituted benzene); MS, m/z 374; Elemental analysis calculated/found (%) C (70.30/70.26), H (4.83/4.68), N (11.18/11.12).

4-phenyl-1-(1,5-diphenylpenta-2,4-dienylidene)semicarbazide **(7)**
1H-NMR (δ/ppm in $CDCl_3$): 7.11-7.64 (m, 15H, Ar-H), 7.69 (s, 1H, –CH=CH–), 7.72 (s, 1H, –CH=CH–), 7.88-8.12 (dd, 2H, –CH=CH–), 8.34 (s, 1H, ArNH, D_2O exchangeable), 9.42 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3450 (NH), 3300–3240 (CONH), 1670 (–CH=CH–),1590 (C-N), 1616, 1558 (aromatic), 754, 697 (monosubstituted benzene); MS, m/z 366; Elemental analysis calculated/found (%) C (78.45/78.26), H (5.76/5.68), N (11.44/11.12).

1-[1-(4-aminophenyl)-3-phenylallylidene]-4-phenylsemicarbazide **(8)**
1H-NMR (δ/ppm in $CDCl_3$): 6.41 (s, 2H, NH_2, D_2O exchangeable), 7.11-7.64 (m, 14H, Ar-H), 7.75 (s, 1H, –CH=CH–), 7.81 (s, 1H, –CH=CH–), 8.41 (s, 1H, ArNH, D_2O exchangeable), 9.64 (s, 1H, CONH, D_2O exchangeable),; IR (KBr/cm^{-1}): 3459 (NH), 3309–3241 (CONH), 1674 (–CH=CH–),1593 (C-N), 1616, 1553 (aromatic), 754, 687 (monosubstituted benzene); MS, m/z 355; Elemental analysis calculated/found (%) C (74.14/74.06), H (5.66/5.58), N (15.72/15.62).

1-[1-(4-aminophenyl)-3-(4-chlorophenyl)allylidene]-4-phenylsemicarbazide **(9)**
1H-NMR (δ/ppm in $CDCl_3$): 6.52 (s, 2H, NH_2, D_2O exchangeable), 7.17-7.65 (m, 13H, Ar-H), 7.72 (s, 1H, –CH=CH–), 7.94 (s, 1H, –CH=CH–), 8.34 (s, 1H, ArNH, D_2O exchangeable), 9.44 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3452 (NH), 3300–3246 (CONH), 1678 (–CH=CH–),1597 (C-N), 1626, 1567 (aromatic), 872 (Cl), 755, 697 (monosubstituted benzene); MS, m/z 389; Elemental analysis calculated/found (%) C (67.60/67.56), H (4.90/4.78), N (14.33/14.26).

4-[4-chlorophenyl-1-(1,3-diphenylallylidene)]semicarbazide **(10)**
1H-NMR (δ/ppm in $CDCl_3$): 7.11-7.64 (m, 14H, Ar-H), 7.71 (s, 1H, –CH=CH–), 7.93 (s, 1H, –CH=CH–), 8.35 (s, 1H, ArNH, D_2O exchangeable), 9.42 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3450 (NH), 3300–3240 (CONH), 1670 (–CH=CH–),1590 (C-N), 1616, 1558 (aromatic), 878 (Cl), 754, 697 (monosubstituted benzene); MS, m/z 374; Elemental analysis calculated/found (%) C (70.30/70.26), H (4.83/4.78), N (11.18/11.12).

4-[4-chlorophenyl-1-(1,5-diphenylpenta-2,4-dienylidene)]semicarbazide **(11)**
1H-NMR (δ/ppm in $CDCl_3$): 7.11-7.64 (m, J= 8.32 Hz, 12H, Ar-H) 7.69 (s, 1H, –CH=CH–), 7.78 (s, 1H, –CH=CH–), 7.87-8.12 (dd, 2H, –CH=CH–), 8.34 (s, 1H, ArNH, D_2O exchangeable), 9.44 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3448 (NH), 3310–3250 (CONH), 1675 (–CH=CH–),1593 (C-N), 1619, 1560 (aromatic), 877 (Cl), 751, 692 (monosubstituted benzene); MS, m/z 400; Elemental analysis calculated/found (%) C (71.73/71.66), H (5.02/4.98), N (10.46/10.12).

4-(2-chlorophenyl)-1-[3-(4-chlorophenyl)-1-phenylallylidene] semicarbazide **(12)**
1H-NMR (δ/ppm in $CDCl_3$): 7.11-7.64 (m, 13H, Ar-H), 7.74 (s, 1H, –CH=CH–), 7.9 (s, 1H, –CH=CH–), 8.35 (s, 1H, ArNH, D_2O exchangeable), 9.43 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3451 (NH), 3300–3247 (CONH), 1678 (–CH=CH–),1597 (C-N), 1613, 1548 (aromatic), 877 (Cl), 756, 696 (monosubstituted benzene); MS, m/z 409; Elemental analysis calculated/found (%) C (64.40/64.26), H (4.18/4.08), N (10.24/10.12).

1-[1-{4-aminophenyl-3-(4-chlorophenyl)}allylidene]-4-(2-chlorophenyl) semicarbazide **(13)**
1H-NMR (δ/ppm in $CDCl_3$): 6.54 (s, 2H, NH_2, D_2O exchangeable), 7.11-7.62 (m, 12H, Ar-H), 7.71 (s, 1H, –CH=CH–), 7.92 (s, 1H, –CH=CH–), 8.33 (s, 1H, ArNH, D_2O exchangeable), 9.44 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3452 (NH), 3308–3241 (CONH), 1672 (–CH=CH–),1593 (C-N), 1616, 1556 (aromatic), 878 (Cl), 758, 695 (monosubstituted benzene); MS, m/z 424; Elemental analysis calculated/found (%) C (62.13/62.06), H (4.27/4.18), N (13.17/13.12).

4-[2-chlorophenyl-1-(1,5-diphenylpenta-2,4-dienylidene)]semicarbazide **(14)**
1H-NMR (δ/ppm in $CDCl_3$): 7.12-7.63 (m, 14H, Ar-H), 7.74 (s, 1H, –CH=CH–), 7.93 (s, 1H, –CH=CH–), 7.97-8.22 (dd, 2H, –CH=CH–), 8.38 (s, 1H, ArNH, D_2O exchangeable), 9.46 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3453 (NH), 3310–3245 (CONH), 1673 (–CH=CH–),1592 (C-N), 1615, 1556 (aromatic), 872 (Cl), 752, 696 (monosubstituted benzene); MS, m/z 400; Elemental analysis calculated/found (%) C (71.73/71.66), H (5.02/4.92), N (10.46/10.33).

4-(4-bromophenyl)-1-(1,3-diphenylallylidene)semicarbazide **(16)**
1H-NMR (δ/ppm in $CDCl_3$): 7.12-7.66 (m, 14H, Ar-H), 7.72 (s, 1H, –CH=CH–), 7.89 (s, 1H, –CH=CH–), 8.39 (s, 1H, ArNH, D_2O exchangeable), 9.47 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3445 (NH), 3310–3243 (CONH), 1674 (–CH=CH–),1592 (C-N), 1615, 1552 (aromatic), 678 (Br), 697 (monosubstituted benzene); MS, m/z 419; Elemental analysis calculated/found (%) C (62.87/62.66), H (4.32/4.28), N (10.00/9.98).

4-(4-bromophenyl)-1-(3-(2-hydroxyphenyl)-1-phenylallylidene)semicarbazide **(17)**
1H-NMR (δ/ppm in $CDCl_3$): 7.12-7.65 (m, 13H, Ar-H), 7.77 (s, 1H, –CH=CH–), 8.01 (s, 1H, –CH=CH–), 8.37 (s, 1H, ArNH, D_2O exchangeable), 9.48 (s, 1H, CONH, D_2O exchangeable), 10.26(s, 1H, OH); IR (KBr/cm^{-1}): 3447 (NH), 3308–3242 (CONH), 1676 (–CH=CH–),1594 (C-N), 1618, 1550 (aromatic), 774 (Br), 697 (monosubstituted benzene); MS, m/z 435; Elemental analysis calculated/found (%) C (60.56/60.48), H (4.16/4.08), N (9.68/9.62).

4-(4-bromophenyl)-1-(3-(4-(dimethylamino)phenyl)-1-phenylallylidene) semicarbazide **(18)**
1H-NMR (δ/ppm in $CDCl_3$): 3.14 (s, 6H, CH_3), 7.10-7.64 (m, 13H, Ar-H), 7.78 (s, 1H, –CH=CH–), 7.96 (s, 1H, –CH=CH–), 8.46 (s, 1H, ArNH, D_2O exchangeable), 9.37 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3456 (NH), 3312–3248 (CONH), 1675 (–CH=CH–),1595 (C-N), 1619 (aromatic), 752 (Br), 693 (monosubstituted benzene); MS, m/z 462; Elemental analysis calculated/found (%) C (62.21/61.98), H (5.00/4.96), N (12.09/11.92).

4-(4-bromophenyl)-1-(3-(4-chlorophenyl)-1-phenylallylidene)semicarbazide **(19)**
1H-NMR (δ/ppm in $CDCl_3$): 7.11-7.67 (m, 13H, Ar-H), 7.78 (s, 1H, –CH=CH–), 7.94 (s, 1H, –CH=CH–), 8.45 (s, 1H, ArNH, D_2O exchangeable), 9.48 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3453 (NH), 3307–3248 (CONH), 1677 (–CH=CH–),1597 (C-N), 1614, 1548 (aromatic), 777 (Br), 696 (monosubstituted benzene); MS, m/z 453; Elemental analysis calculated/found (%) C (58.11/58.06), H (3.77/3.74), N (9.24/9.14).

4-(4-bromophenyl)-1-(1,5-diphenylpenta-2,4-dienylidene)semicarbazide **(20)**
1H-NMR (δ/ppm in $CDCl_3$): 7.10-7.64 (m, 14H, Ar-H), 7.74 (s, 1H, –CH=CH–), 7.93 (s, 1H, –CH=CH–), 7.98-8.21 (dd, 2H, –CH=CH–), 8.58 (s, 1H, ArNH, D_2O exchangeable), 9.47 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3452 (NH), 3310–3255 (CONH), 1675 (–CH=CH–),1597 (C-N), 1614, 1553 (aromatic), 772 (Br), 752, 696 (monosubstituted benzene); MS, m/z 445; Elemental analysis calculated/found (%) C (64.58/64.46), H (4.52/4.42), N (9.41/9.38).

4-(4-bromophenyl)-1-(1-(4-hydroxyphenyl)-3-phenylallylidene)semicarbazide **(21)**
1H-NMR (δ/ppm in $CDCl_3$): 7.12-7.65 (m, 13H, Ar-H), 7.76 (s, 1H, –CH=CH–), 7.9 (s, 1H, –CH=CH–), 8.36 (s, 1H, ArNH, D_2O exchangeable), 9.43 (s, 1H, CONH, D_2O exchangeable), 10.42 (s, 1H, OH); IR (KBr/cm^{-1}): 3455 (NH), 3305–3248 (CONH), 1679 (–CH=CH–),1594 (C-N), 1613, 1546 (aromatic), 776 (Br), 752, 694 (monosubstituted benzene); MS, m/z 435; Elemental analysis calculated/found (%) C (60.56/60.28), H (4.16/4.12), N (9.63/9.54).

4-(4-bromophenyl)-1-(3-(4-(dimethylamino)phenyl)-1-(4-hydroxyphenyl) allylidene)semicarbazide **(22)**
1H-NMR (δ/ppm in $CDCl_3$): 3.45 (s, 6H, CH_3), 7.12-7.68 (m, 12H, Ar-H), 7.86 (s, 1H, –CH=CH–), 7.92 (s, 1H, –CH=CH–), 8.46 (s, 1H, ArNH, D_2O exchangeable), 9.37 (s, 1H, CONH, D_2O exchangeable), 10.38 (s, 1H, OH); IR (KBr/cm^{-1}): 3448 (NH), 3311–3247 (CONH), 1680 (–CH=CH–),1589 (C-N), 1615, 1558 (aromatic), 757 (Br), 693 (monosubstituted benzene); MS, m/z 478; Elemental analysis calculated/found (%) C (60.13/59.98), H (4.84/4.78), N (11.69/11.62).

Pharmacology

Anti-inflammatory [10-25]

The results of the anti-inflammatory screening of the synthesized compounds are shown in table 2. Animals were divided into control, standard and different test groups comprising of six 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 [24-25]. 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 ip. Administration of the compounds and paw volume was measured plethysmometrically at 0, 1, 2 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 statically by student t test (Dunnett). Mean defference in paw volume was measured and percentage inhibition was calculated by using formula

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

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

Analgesic activity

The peripheral analgesic activity [10-25] was evaluated using acetic acid-induced writhing test in mice and the results are shown in table 3. In this method, Swiss albino mice of either sex weighing between 25-30 gm were randomly distributed in 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

$$\% \text{ protection} = (Nc - Nt / Nc) \times 100$$

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

Table 2: Anti-inflammatory activity of title compounds

Com code	Dose (mg/kg)	Time (hrs)	Thickness variation	% inhibition
Control		1	0.2563±0.0119	-
		2	0.35±0.01768	-
		3	0.4125±0.0125	-
Diclofenac sodium	25	1	0.1563±0.0257*	39.01
		2	0.1375±0.05154**	60.71
		3	0.06875±0.03287**	83.33
4	30	1	0.1563±0.01197*	39.01
		2	0.1313±0.02954**	62.48
		3	0.0250±0.0**	64.93
5	30	1	0.1563±0.01197*	39.01
		2	0.1313±0.02954**	62.48
		3	0.0250±0.0**	63.93
6	30	1	0.2188±0.09862	14.63
		2	0.2163±0.06722	47.61
		3	0.1125±0.03146**	71.87
7	30	1	0.1913±0.05242*	25.36
		2	0.2138±0.03287**	25.39
		3	0.1375±0.03608**	65.62
8	30	1	0.1997±0.03125	22.08
		2	0.1989±0.00625**	66.05
		3	0.0875±0.0125**	78.78
9	30	1	0.1775±0.02165	30.70
		2	0.1475±0.03146*	46.42

		3	0.1±0.02041**	75.75
10	30	1	0.1988±0.03590	22.43
		2	0.1788±0.02577	31.74
		3	0.08125±0.01573**	79.68
11	30	1	0.1563±0.01197*	39.01
		2	0.1313±0.02954**	62.48
		3	0.0250±0.0**	78.93
12	30	1	0.1313±0.006250**	48.77
		2	0.08125±0.01875**	76.78
		3	0.03125±0.01197**	92.42
13	30	1	0.1575±0.04732**	38.54
		2	0.1263±0.02772**	69.62
		3	0.0375±0.0125**	90.9
14	30	1	0.1763±0.04002	31.21
		2	0.1375±0.01197**	73.21
		3	0.0375±0.0125**	90.9
15	30	1	0.1563±0.01197*	39.01
		2	0.1313±0.02954**	62.48
		3	0.0250±0.0**	93.93
16	30	1	0.1563±0.01197*	39.01
		2	0.1313±0.02954**	62.48
		3	0.0250±0.0**	80.93
17	30	1	0.1563±0.01197*	39.01
		2	0.1313±0.02954**	62.48
		3	0.0250±0.0**	86.93
18	30	1	0.2000±0.05951*	21.96
		2	0.1668±0.01443*	55.56
		3	0.075±0.02887**	81.25
19	30	1	0.1563±0.0257*	39.01
		2	0.1375±0.05154**	60.71
		3	0.06875±0.03287**	83.33
20	30	1	0.1563±0.01197*	39.01
		2	0.1313±0.02954**	62.48
		3	0.0250±0.0**	83.93
21	30	1	0.1563±0.01197*	39.01
		2	0.1313±0.02954**	62.48
		3	0.0250±0.0**	90.23
22	30	1	0.1968±0.03590*	23.21
		2	0.1575±0.04254**	80.75
		3	0.05625±0.03287**	86.36

a) Number of animals in each group n = 6.
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.

Table 3: Analgesic activity of title compounds

Compound	Dose(mg/kg)	Number of writhings (mean ± SEM)	Activity (%)
Control	--	83 ± 6.72	----
Aspirin	50	18 ± 2.66**	78.31
4	30	22.80 ± 5.21*	72.53
5	30	31 ± 4.46	62.65
6	30	23.01 ± 5.58**	75.90
7	30	34.5 ± 4.46	58.43
8	30	17.12 ± 4.41**	78.37
9	30	18 ± 2.66**	78.31
10	30	23.8 ± 7.59*	71.32
11	30	25.8 ± 7.59*	68.91
12	30	42.5 ± 5.12*	48.79
13	30	16.1±3.41**	80.60
14	30	24.2±2.86*	70.84
15	30	29.62±4.11**	64.31
16	30	23±1.98**	75.90
17	30	12.5 ± 4.48**	84.93
18	30	27.80±3.11*	66.50
19	30	24.7±4.16**	70.24
20	30	31.2±2.23*	62.40
21	30	14 ± 3.10**	83.13
22	30	16.52±3.21*	80.9

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.

Statistics
The results are expressed as the mean ± SEM of six animals per group. The data were statistically analyzed by one-way analysis of Variance (ANOVA) followed by posthoc. test. Differences with $P < 0.05$ between experimental groups were considered statistically significant.

RESULTS AND DISCUSSION

The anti-inflammatory and analgesic activity of the synthesized compounds is summarized in Table 2 and Table 3 respectively. As from the tables it could be seen that most of the compounds showing anti-inflammatory and analgesic activity more or comparable to the reference drugs. Comparison of the anti-inflammatory activity of all tested compounds revealed that compound 15 was the most active compound in the synthesized compounds. The order of activity regarding aniline substitution is NO_2>o-Cl>p-Br>unsubstituted aniline>p-Cl. As can be seen from Table 2, the halo substituted aniline compounds were more potent anti-inflammatory agents than the other synthesized compounds, may be due to the increase of lipophilicity, which may lead to increases

in bioavailability. But in the case of p-Cl substituted aniline, the substitution disfavors the activity. Among the synthesized compounds, compound 6, 12, 13, 14, 15, 19, 20 and 21 showed the better activity in comparison to diclofenac sodium as the reference drug. In reference to the substitution on the acetophenic phenyl in chalcone moiety, the unsubstitution (6, 12, 14 and 16) is comparatively favorable than the substitution (13) for activity. The lengthing of the carbon chain i.e. cinnameldehyde (7, 11, 14, 15 and 20) is more favorable than simple aldehydic carbon chain. The substitution on phenyl group in aldehydic group is favorable for the activity (6, 12, 13 and 19) than the unsubstitution (10, 16). But in case of bulky substitution (5, 18 and 22) the substitution decrease the activity, may be due to the improper attachment with the receptor. The hydroxyl substituted compounds (21) showing the good activity, may be due to the increased hydrogen bonding, which leads to the proper attachment with receptor level. When both substitutions were present (22) one (OH) favors the attachment and the other one ($(CH_3)_2N$) disfavors the attachment. So the resulting activity is moderate not good. These observations clarify the role of type of substitutions, helps to increase the activity.

But in case of analgesic activity only some compounds (12, 13, 14, 15 and 20) showed the observation comparable to the anti-inflammatory activity. Most of the compounds gave the different observation. Compound 12, 13, 14 and 20 are more potent than the standard drug asprin. In case of analgesic activity the bromine substitution on the phenyl group of aniline is more favorable than any other substitution and if hydroxyl group is also present in the chalcone moiety with the bromine substitution it give the more favorable result (2, 3), may be due to increased hydrogen bonding. It can also be concluded from the observed activity of the compound 8 and 9.

In summary, most of the synthesized compounds were potential lead for an anti-inflammatory and analgesic activity. On the bases of observed results, it may be concluded that the substitution favors the activity, but the bulkier substitution may also disfavors the activity, may be due to the improper attachment with binding site. The hydrogen bonding is also play an important role in the pharmacological activity especially for analgesic activity.

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