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SYNTHESIS OF THE 2,4-DIPHENYL-5-IMINO-Δ^2-1,3,4-THIADIAZOLE DERIVATIVES AS POTENTIAL ANTINOCICEPTIVE COMPOUNDS

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In this study, some new derivatives of 2,4-diphenyl-5-imino-Δ^2-1,3,4-thiadiazole were prepared by reacting α-chlorobenzal phenylhydrazone derivatives ***(2a–2h)*** *with potassium thiocyanate to yield 2,4-diphenyl-5-imino-Δ^2-1,3,4-thiadiazole derivatives* ***(3a–3h).*** *The structures of the compounds were elucidated by IR, ^{1}H-NMR and elemental analysis. Antinociceptive activity was evaluated using in vivo tests, by means of the hot plate method. Qualitatively, all the compounds exerted a lesser antinociceptive effect compared to acetylsalicylic acid.*

Keywords: *Antinociceptive activity, Hydrazonyl, Thiadiazole, Hot plate method*

INTRODUCTION

Non-steroidal antiinflammatory drugs (NSAIDs) belong to a variety of chemical classes with no common features except for the absence of a steroidal structure. Their primary effect is pain relief but they also possess antipyretic and antiinflammatory activities. Their pro-algogenic effects are explained by sensitisation of nociceptive nerve endings to the stimulating effect (algogenic) of kinins (bradykinin), serotonin and histamine. In addition, production of prostanoid in the brain has a thermoregulatory effect. Prostaglandins (PGs) sensitise peripheral nerve endings and nociceptors to transmit pain signals to the brain and the spinal cord (Dannhardt and Kiefer 2001).

Despite the availability of a number of NSAIDs, suppression of pain and inflammation still continues to be a challenge. This is because NSAIDs do not only exhibit a different spectrum of analgesic, antipyretic and antiinflammatory effects but also cause gastrointestinal (GI) complications ranging from dyspepsia to fatal upper GI tract bleeding and perforation. Efforts to improve the adverse effect profile of the current NSAIDs have been focused on developing new drugs, prodrugs or modifications of marketed formulations. There are over 20 NSAIDs regularly prescribed in the clinics with undesirable side effects; hence the search for newer drugs (Duffy, Dearden and Rostron 2001).

The biological profile of thiadiazoles is very extensive (Kulkarni, Sajjan and Lagali 1992; Arun, Nag and Panda 1999). 1,3,4-thiadiazole derivatives are associated with diverse biological activities probably by virtue of toxophoric -N=C-S- grouping. The reported biological activities of 1,3,4-thiadiazoles include antiinflammatory, antiviral, leishmenicidal, adenosine receptor antagonist, diuretic activity, antihypertensive,

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anthelmintic, antifungal, antimicrobial and analgesic activity. As a result, many new substituted 1,3,4-thiadiazole derivatives were developed (Turner *et al.* 1988; Stephen *et al.* 1988; Moronfolu and Michael 1989; Marin *et al.* 1992; Firoozi *et al.* 1995; Shafiee *et al.* 1995; Shafiee and Foroumadi 1996; Foroumadi, Daneshtalab and Shafiee 1999; Alireza *et al.* 2001; Foroumadi, Mirzaei and Shafiee 2001; Foroumadi, Kiani and Soltani 2003; Emami and Foroumadi 2005, Foroumadi *et al.* 2005a,b, 2004, 2002; El Ashry *et al.* 2006; Trotsko, Dobosz and Jagiello-Wojtowicz 2007; Onkol *et al.* 2008; Rajesh, Jitendra and Subhash 2008).

The synthesis and studies on the analgesic properties of compounds containing substituted 1,3,4-thiadiazoles have been described previously (Song *et al.* 1999; Tijen, Bilge and Fethi 2004; Adnan *et al.* 2007; Gadad *et al.* 2008; Amir, Kumar and Javed 2007, 2006; Onkol *et al.* 2008). Stimulated by these findings, our attention has been focused on the synthesis of a series of new 1,3,4-thiadiazole derivatives, which are expected to show antinociceptive activity.

METHODS

Experimental

All chemicals and solvents used were of reagent grade (Merck, Loba Chem or CDH), and were used without further purification. The identification of test compounds in the initial stages of the experiment was done by the TLC method and the most common solvent systems used were toluene, ethyl acetate, and formic acid in the ratio of 5:4:1, and benzene and acetone in 5:1 and 4:1 ratios. Melting points were recorded in open capillary tubes in liquid paraffin and are uncorrected. IR spectra were recorded by using KBr pellet technique on Perkin Elmer spectrometer. ^{1}HNMR spectra were recorded in deuterated chloroform using TMS as internal reference on Bruker Avance 400 NMR spectrometer. Elementary analyses were performed on a Leco CHNS 932 analyser and satisfactory results of calculated values (C, H, N) were obtained.

The compounds were synthesised as shown in Figure 1. Substituted hydrazonoyl **(1a–1h)** were synthesised from reaction between benzoyl chloride and phenyl hydrazine derivatives. Substituted hydrazonoyl **(1a–1h)** derivatives were chlorinated to from α-chlorobenzal phenylhydrazone derivatives **(2a–2h)**. Cyclisation of α-chlorobenzal phenylhydrazone derivatives **(2a–2h)** with potassium thiocyanate (KCNS) yielded 2,4-diphenyl-5-imino-Δ^2-1,3,4-thiadiazole derivatives **(3a–3h)**.

*General procedure of substituted hydrazonoyl **(1a–1h)** synthesis*

Benzoyl chloride (0.01 M) or its derivatives were dissolved in methanol and ethanol (10 to 25 mL). Pyridine (0.005 M) was added along with phenyl hydrazine (0.01 M) or its derivatives. The reaction mixture was refluxed at 50°C–60°C for 2 to 15 h depending on the derivative used. The reaction time was monitored by TLC. Various hydrazonoyl derivatives **(1a–1h)** were obtained and the products were recrystallised by ethanol.

General procedure of substituted a-chlorobenzal phenylhydrazone derivatives ***(2a–2h)*** *synthesis*

The substituted hydrazonoyl (0.01 M) and its derivatives **(1a–1h)** were chlorinated using phosphorus pentachloride, PCl_5 (0.01 M) in methanol and ethanol. It was then refluxed for 5 to 12 h at 40°C–60°C depending on the different derivatives used in the reaction. The reaction time was monitored by TLC. Various α-chlorobenzal hydrazone derivatives **(2a–2h)** were obtained after keeping the mixture overnight. The compounds **(2a–2h)** were recrystallised by ethanol.

General procedure of substituted 2,4-diphenyl-5-imino-Δ²-1,3,4-thiadiazole derivatives ***(3a–3h)*** *synthesis: Cyclisation*

The reaction of substituted α-chlorobenzal phenylhydrazone (0.003 M) derivatives was carried out with KCNS (0.005 M) in methanol. The reaction mixture was refluxed at 50°C–70°C for 3 to 10 h. The reaction time was monitored by TLC. When the reaction was complete, the mixture was kept overnight. The compounds **(3a–3h)** were then recrystallised with ethanol.

Synthesis of 2,4-diphenyl-5-imino-Δ²-1,3,4-thiadiazole ***(3a)***

Yield 38%, m.p. 200°C, molecular formula $C_{14}H_{11}N_3S$, formula mass (g/mol) 253.32, elemental analysis (%) found: H (4.37), C (66.37), N (16.58), S (12.65), calculated: H (4.31), C (66.42), N (16.49), S (12.58). IR spectra cm^{-1}: 1645cm^{-1} (C=O), 3208 cm^{-1} (NH), 1599 cm^{-1} (C-C, Ar), 582 cm^{-1} (C-Cl), 1682 cm^{-1} (C=N), 3201 cm^{-1} (NH), 692 cm^{-1} (C-S-C), 1601 cm^{-1} (C=N), 3391 cm^{-1} (NH). ^1H NMR spectra (DMSO-d_6) ppm: 6.8–7.34 (10H, m, Ar-H), 8.32 (1H, s, NH imine).

Synthesis of 2-phenyl-2-(2´,4´-dinitrophenyl)-5-imino-Δ²-1,3,4-thiadiazole ***(3b)***

Yield 75%, m.p. 202°C, molecular formula $C_{14}H_9N_5O_4S$, formula mass (g/mol) 343.32, elemental analysis (%), found: H (2.64), C (48.97), N (20.39), S (9.33), calculated: H (2.61), C (48.93), N (20.38), S (9.40). IR spectra cm^{-1}: 1519 cm^{-1} (NO_2), 661 cm^{-1} (C-S-C), 1661 cm^{-1} (C=N), 3374 cm^{-1} (NH). ^1H NMR spectra (DMSO-d_6) ppm: 7.3–8.2 (5H, m, Ar), 10.9 (1H, s, NH), 8.3–9.14 (3H, m, Ar-NO_2).

Synthesis of 2-(2´-chlorophenyl)-4-phenyl-5-imino-Δ²-1,3,4-thiadiazole ***(3c)***

Yield 57%, m.p. 198°C, molecular formula $C_{14}H_{10}N_3SCl$, formula mass (g/mol) 287.77, elemental analysis (%), found: H (3.50), C (58.43), N (14.60), S (11.14), calculated: H (3.52), C (58.37), N (14.67), S (11.21). IR spectra cm^{-1}: 670 cm^{-1} (C-S-C), 1595 cm^{-1} (C=N), 3440 cm^{-1} (NH). ^1H NMR spectra (DMSO-d_6) ppm: 7.1–7.8 (9H, m, Ar), 3.7 (1H, s, NH).

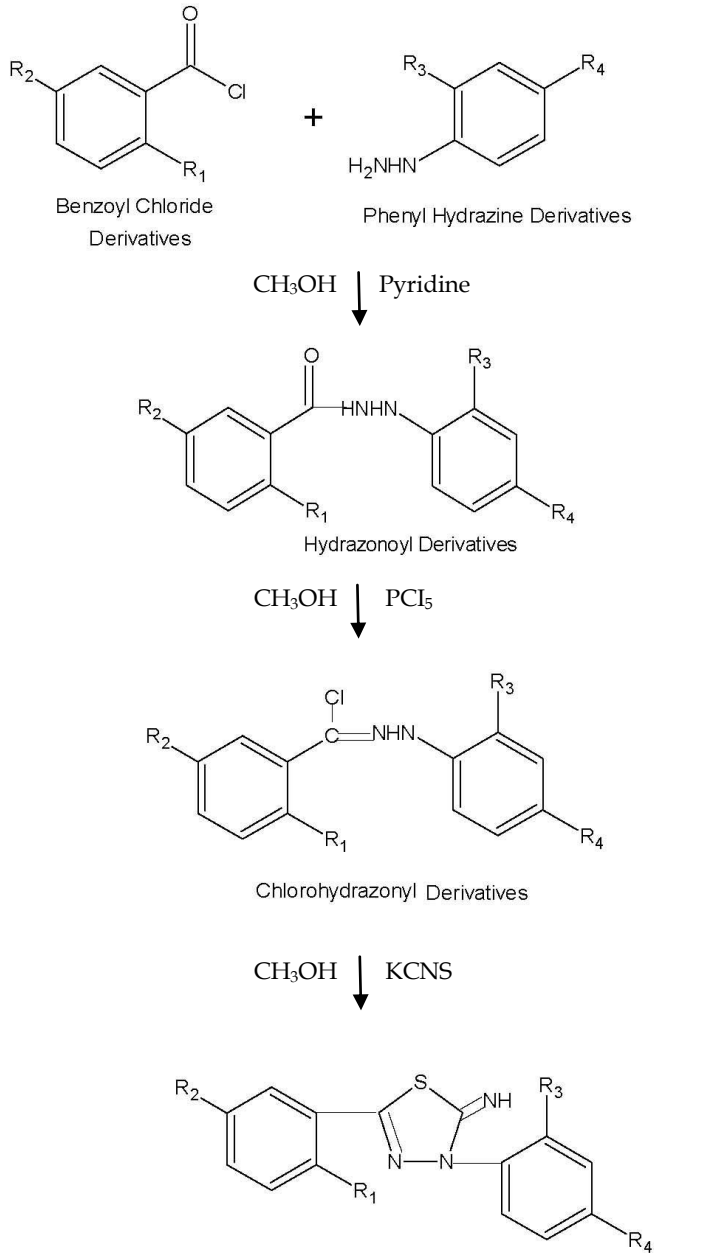

Fig. 1: Synthesis of 1,3,4-thiadiazole derivatives.

Synthesis of 2-[2´-chlorophenyl]-4-(2´´,4´´-dinnitro-phenyl-5-imino-Δ^2-1,3,4-thiadiazole ***(3d)***

Yield 53%, m.p. 164°C, molecular formula $C_{14}H_8O_4N_5SCl$, formula mass (g/mol) 377.76, elemental analysis (%) found: H (2.13), C (44.51), N (18.53), S (8.48), calculated: H (2.16), C (44.43), N (18.48), S (8.52). IR spectra cm^{-1}: 672 cm^{-1} (C-S-C), 1524 cm^{-1} (C=N), 1340 cm^{-1} (NO_2), 3450 cm^{-1} (NH). ^1H NMR (DMSO-d_6) ppm: 8.2–9.0 (4H, m, Ar-Cl), 9.3 (1H, s, NH), 8.3–9.14 (3H, m, Ar-NO_2).

Synthesis of 2-(4´-chlorophenyl)-4-phenyl-5-imino-Δ^2-1,3,4-thiadiazole ***(3e)***

Yield 62%, m.p. 147°C, molecular formula $C_{14}H_{10}N_3SCl$, formula mass (g/mol) 287.77, elemental analysis (%) found: H (3.50), C (58.43), N (14.60), S (11.14), calculated: H (3.45), C (58.35), N (14.54), S (11.17). IR spectra cm^{-1}: 1630 cm^{-1} (C=O), 850 cm^{-1} (Ar-Cl), 3400 cm^{-1} (NH), 820 cm^{-1} (Ar-Cl), 570 cm^{-1} (C-Cl), 1520 cm^{-1} (C=N), 3600 cm^{-1} (NH). ^1H NMR (DMSO-d_6) ppm: 7.2–7.9 (9H, m, Ar), 3.9 (1H, s, NH).

Synthesis of 2-(4´-chlorophenyl)-4-(2´´,4´´-dinitrophenyl)-5-imino-Δ^2-1,3,4-thiadiazole ***(3f)***

Yield 66%, m.p. 210°C, molecular formula $C_{14}H_8O_4N_5SCl$, formula mass (g/mol) 377.76, elemental analysis (%) found: H (2.13), C (44.51), N (18.53), S (8.48), calculated: H (2.15), C (44.47), N (18.57), S (8.43). IR spectra cm^{-1}: 672 cm^{-1} (C-S-C), 1351 cm^{-1} (NO_2), 1595cm^{-1} (C=N), 769 cm^{-1} (Ar-Cl), 3433 cm^{-1} (NH). ^1H NMR (DMSO-d_6) ppm: 7.1–7.8 (4H, m, Ar-Cl), 8.2–8.9 (3H, m, Ar-NO_2).

Synthesis of 2-p-aminophenyl-4-phenyl-5-imino-Δ^2-1,3,4-thiadiazole ***(3g)***

Yield 61%, m.p. 238°C, molecular formula $C_{14}H_{12}N_4S$, formula mass (g/mol) 268.34, elemental analysis (%) found: H (4.50), C (62.66), N (20.87), S (11.94), calculated: H (4.53), C (62.61), N (20.83), S (11.87). IR spectra cm^{-1}: 1398 cm^{-1} (Ar-NH_2), 1631 cm^{-1} (C=N), 3442 cm^{-1} (NH), 692 cm^{-1} (C-S-C).

Synthesis of 2-(p-aminophenyl)-4-(2´´,4´´-dinitrophenyl)-5-imino-Δ^2-1,3,4-thiadiazole ***(3h)***

Yield 56%, m.p. 252°C, molecular formula $C_{14}H_{10}N_6O_4S$, formula mass (g/mol) 358.33, elemental analysis (%) found: H (2.81), C (46.92), N (23.45), S (8.94), calculated: H (2.76), C (46.84), N (23.54), S (8.98). IR spectra cm^{-1}: 1350 cm^{-1} (NO_2), 1595 cm^{-1} (C=N), 671 cm^{-1} (C-S-C), 3443 cm^{-1} (NH). ^1H NMR (DMSO–d_6) ppm: 7.3–7.6 (4H, m, Ar-NH_2), 7.7–7.9 (3H, m, Ar-NO_2), 8.7 (1H, s, NH), 4.3 (1H, s, NH_2).

Preparation of Test Samples for Bioassay

Test samples (50 mg/kg), suspended in 0.5% carboxymethyl cellulose (CMC) in distilled water, were given intraperitoneally (i.p.) to the experimental animals. Control animals received the same experimental handling as the test groups with the exception that the drug treatment was replaced with an appropriate volume of the dosing vehicle (0.5% CMC, 10 mL/kg). Acetyl salicylic acid (50 mg/kg) in 0.5% CMC was used as the standard drug.

Experimental Animals

Albino mice of both sexes (25 to 35 g) were maintained under controlled conditions of light (12 h) and temperature 25 ± 1°C in the Animal House of GRD (PG) IMT, Dehradun, 2 weeks prior to the experiment for acclimatisation. Animals had access to food and water *ad libitum*. All pharmacological activities were carried out as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) norms (Regn No: 1145/a/07/CPCSEA), after obtaining the approval from the Institutional Animal Ethics Committee of Department of Pharmacy, GRD (PG) Institute of Management & Technology, India.

Experimental Design

50 albino mice of either sex were taken and divided into 10 groups with each group consisting of 5 mice. Drug was administered to all groups (control, standard and test) through the i.p. route. Sodium CMC did not produce any evident response in the mice's activity. Group I (control group) was given sodium CMC (0.5% CMC) in distilled water (10 mL/kg body weight). Group II (standard group) was given acetyl salicylic acid (50 mg/kg) suspension in 0.5% sodium CMC at 5 mL/kg body weight. Groups III to X (test groups) were given compounds **3a** to **3h** (test drugs) suspension in 0.5% sodium CMC (50 mg/kg) at 5 mL/kg body weight.

Antinociceptive Activity

Heat was used as a source of pain and the method used was first described by Eddy and Leimbach (1953). Animals were individually placed on a hot plate maintained at a constant temperature (55°C). The reaction of animals, such as paw licking or jump responses was taken as the end response (a cut-off period of 15 sec was observed to avoid damage to the paw). The reaction time of animals on the hot plate at 10, 20, 30, 40 and 50 min after administration of drugs (standard, control and test) in each group were noted. In this study techno analgesic heated plate apparatus was used.

Statistical Analysis

Results were expressed as mean ± S.E.M. Statistical significance was analysed using the one-way analysis of variance followed by Tukey's Multiple Comparison Test where $p<0.05$ was accepted to be of significant difference.

RESULTS AND DISCUSSION

Synthetic Work

The rate of a reaction is affected by the solvent used because of the varying abilities of solvents to solvate reagents and transition state. Solvation refers to specific interactions between solvent molecules and dissolved reagents and or transition state. These

interactions are hydrogen bonding, dipole-dipole and ion-dipole interactions. Hence, the formation of products and the rate of reaction were affected by the solvent used.

The structure of the compounds was elucidated by FT-IR, ^{1}H-NMR and elemental analyses. Crystallisation solvents, melting points (M.P.), yield (%), elemental analyses and spectral data of the compounds (**1a–1h** and **2a–2h**) are given in Table 1. Table 1 shows that all derivatives gave moderate to good yield (38%–76%). The FT-IR spectra of 1,3,4-thiadiazoles showed the characteristic bonds at 670, 1600 and 3400 cm^{-1} for C-S-C-,-C=N-, and NH (imino) groups, respectively. In the ^{1}H-NMR spectra, all protons were in accordance with the expected chemical shift and integral values. Aromatic protons of aryl substituted 1,3,4-thiadiazoles derivatives **(3a–3h)** were seen at 6.8–8.0 ppm and singlet chemical shift of amino NH at 8.0–9.0 ppm. Table 2 shows 2,4-diphenyl-5-imino-Δ^2-1,3,4-thiodiazole derivatives and its respective side chains.

Among the derivatives of substituted 2,4-diphenyl-5-imino-Δ^2-1,3,4-thiadiazole, compound **3c** showed more analgesic activity than others (Table 3). In this compound, substitution of 1,3,4-thiadiazole by 2′-chlorophenyl was at position 2, and phenyl at position 4. Compounds **3f** and **3h** showed less analgesic action than **3c.** Compound **3f** had 4′-chlorophenyl at position 2 and 2″,4″-dinitrophenyl at position 4. Compound **3h** was substituted with 4′-aminophenyl at position 2 and 2″,4″-dinitrophenyl at position 4 of the 1,3,4-thiadiazole, respectively (Table 2). Some evidences have suggested that the hydrazone moiety present in the derivative possesses different pharmacophoric characters for their pharmacological effects. According to these results, analgesic profile of a new series of 2,4-diphenyl-5-imino-Δ^2-1,3,4-thiadiazole derivatives **(3a–3h)** contained -N-N=C in heterocyclic ring (Salgin-Göken *et al.* 2007; Olayinka *et al*. 2009). Thus, we decided to substitute thiadiazole moiety with phenyl groups at positions 2 and 4, in the hope of obtaining additional inhibitors of cyclooxygenase enzymes (COX). Two substituted phenyl groups were present at positions 2 and 4 in the 5-imino-1,3,4-thiadiazole ring, and both phenyl rings were substituted by electrophilic (Cl, NO_2) and nucleophilic (NH_2) groups (Table 2). Our results showed that, electrophilic substitution of phenyl ring is beneficial for antinociceptive activity. Therefore, it is possible that replacement of these kinds of aryl groups with a thiadiazole structure has changed the mechanism of enzyme-receptor interaction and highlighted the importance of aryl rings substituents. Since in vivo activity depends on highly complex physiological interactions, at this moment we are unable to rationalise all of these pharmacological results.

Pharmacology

Antinociceptive activity testing of the synthesised compounds was carried out utilising the hot plate method. All compounds were found to be giving less antinociceptive activity than acetyl salicylic acid. Compound **3c** showed the highest activity. Acetyl salicylic acid offers relief from inflammatory pain by suppressing the formation of pain substances in the peripheral tissues, where prostaglandins and bradykinin were suggested to play an important role in the pain process (Hirose *et al.* 1984). NSAIDs inhibit COX-1 and COX-2 which catalyse the conversion of arachidonic acid to PGs and thus prevent the formation of PGs. COX-1 is a constitutive isoform and is found in the G1 tract, the kidney and platelets, and is believed to be responsible for the maintenance of physiological homeostasis such as G1 integrity and renal function. On the other hand, COX-2 is induced by many kinds of inflammatory mediators, and plays an important role in the

prostaglandin biosynthesis associated with inflammatory responses. Inhibition of both COX-1 and COX-2 by classical NSAIDs leads to a decrease in all PG synthesis, which accounts for the beneficial antiinflammatory and analgesic effects of NSAIDs as well as the G1 side effects (Dannhardt and Laufer 2000). Therefore, it is likely that compounds **3a–3h** might suppress the formation of these substances or antagonise the action of these substances and thus exerts its analgesic activity. Therefore, our initial screening results demonstrated that the presence of electrophilic substituted phenyl group on 2,4-di-phenyl-5-imimno-1,3,4-thiadiazole ring might contribute to their analgesic activity. Further studies using these compounds might be useful to develop better candidates with potent analgesic activity

CONCLUSION

A series of 2,4-diphenyl-5-imino-Δ^2-1,3,4-thiadiazole derivatives were successfully prepared and they demonstrated good antinociceptive activity. The most promising compound with antinociceptive activity was 2-(2´-chlorophenyl)-4-phenyl-5-imino-Δ^2-1,3,4-thiadiazole. Further studies are needed to shed light on the mechanisms of action of these compounds.

Table 1: Physicochemical data for the synthesised compounds.

Compound no.	Yield (%)	Molecular formula	Formula mass (g/mol)	Elemental analysis (%)		
				Element found		Calculated
1a	76	$C_{13}H_{12}ON_2$	212.25	H	5.69	5.73
				C	73.56	73.61
				N	13.19	13.43
1b	67	$C_{13}H_{10}N4O_5$	302.24	H	3.33	3.41
				C	51.66	51.56
				N	18.53	18.56
1c	45	$C_{13}H_{11}N_2OCl$	246.5	H	4.49	4.43
				C	63.29	63.24
				N	11.35	11.41
1d	66	$C_{13}H_9O_5N_4Cl$	336.69	H	2.69	2.64
				C	46.37	46.33
				N	16.64	16.43
1e	62	$C_{13}H_{11}N_2OCl$	246.569	H	4.49	4.44
				C	63.29	63.21
				N	11.35	11.37

(continued on next page)

Table 1: *(continued)*

Compound no.	Yield (%)	Molecular formula	Formula mass (g/mol)	Elemental analysis (%)		
				Element found		Calculated
1f	65	$C_{13}H_9O_5N_4Cl$	336.69	H	2.69	2.62
				C	46.37	46.32
				N	16.64	16.60
1g	53	$C_{13}H_{13}ON_3$	227.26	H	16.64	16.61
				C	68.70	68.75
				N	18.48	18.41
1h	59	$C_{13}H_{11}N_5O_5$	317.26	H	3.49	3.45
				C	49.21	49.25
				N	22.07	22.03
2a	47	$C_{13}H_{11}N_2Cl$	230.69	H	4.80	4.86
				C	67.68	67.62
				N	12.14	12.10
2b	73	$C_{13}H_9N_4O_4Cl$	320.69	H	2.82	2.78
				C	48.68	48.71
				N	17.47	17.43
2c	79	$C_{13}H_{10}N_2C_{l2}$	265.14	H	3.80	3.74
				C	58.89	58.94
				N	10.56	10.54
2d	56	$C_{13}H_8O_4N_4Cl_2$	355.13	H	2.27	2.32
				C	43.96	43.85
				N	15.77	15.73
2e	47	$C_{13}H_{10}N_2C_{l2}$	265.14	H	3.80	3.86
				C	58.89	58.84
				N	10.56	10.51
2f	63	$C_{13}H_8O_4N_4Cl_2$	355.13	H	2.27	2.34
				C	43.96	43.90
				N	15.77	15.71
2g	51	$C_{13}H_{12}N_3Cl$	245.71	H	4.92	4.86
				C	63.54	63.49
				N	17.10	17.17
2h	71	$C_{13}H_{10}N_5O_4Cl$	335.7	H	3.00	3.44
				C	46.51	46.55
				N	20.86	20.81
3a	38	$C_{14}H_{11}N_3S$	253	H	4.37	4.31
				C	66.37	66.42
				N	16.58	16.49
				S	12.65	12.58

(continued on next page)

Table 1: *(continued)*

Compound no.	Yield (%)	Molecular formula	Formula mass (g/mol)	Elemental analysis (%)		
				Element found		Calculated
3b	75	$C_{14}H_9N_5O_4S$	343	H	2.64	2.61
				C	48.97	48.93
				N	20.39	20.38
				S	9.33	9.40
3c	57	$C_{14}H_{10}N_3SCl$	287.5	H	3.50	3.52
				C	58.43	58.37
				N	14.60	14.67
				S	11.14	11.21
3d	53	$C_{14}H_8O_4N_5SCl$	377.5	H	2.13	2.16
				C	44.51	44.43
				N	18.53	18.48
				S	8.48	8.52
3e	62	$C_{14}H_{10}N_3SCl$	287.5	H	3.50	3.45
				C	58.43	58.35
				N	14.60	14.54
				S	11.14	11.17
3f	66	$C_{14}H_8O_4N_5SCl$	377.5	H	2.13	2.15
				C	44.51	44.47
				N	18.53	18.57
				S	8.48	8.43
3g	61	$C_{14}H_{12}N_4S$	268	H	4.50	4.53
				C	62.66	62.61
				N	20.87	20.83
				S	11.94	11.94
3h	56	$C_{14}H_{10}N_6O_4S$	294	H	2.81	2.76
				C	46.92	46.84
				N	23.45	23.54
				S	8.94	8.98

Table 2: 2,4-diphenyl-5-imino-Δ^2-1,3,4-thiadiazole derivatives.

Compounds	R_1	R_2	R_3	R_4
2,4-diphenyl-5-imino-Δ^2-1,3,4-thiadiazole **(3a)**	H	H	H	H
2-phenyl-2-(2´,4´-initrophenyl)-5-imino-Δ^2-1,3,4-thiadiazole **(3b)**	H	H	NO_2	NO_2
2-(2´-chlorophenyl)-4-phenyl-5-imino-Δ^2-1,3,4-thiadiazole **(3c)**	Cl	H	H	H
2-(2´-chlorophenyl)-4-(2´´,4´´-dinnitro-phenyl-5-imino- Δ^2-1,3,4-thiadiazole **(3d)**	Cl	H	NO_2	NO_2
2-(4´-chlorophenyl)-4-phenyl-5-imino-Δ^2-1,3,4-thiadiazole **(3e)**	H	Cl	H	H
2-(4´-chlorophenyl)-4-(2´´,4´´-dinitrophenyl)-5-imino-Δ^2-1,3,4-thiadiazole **(3f)**	H	Cl	NO_2	NO_2
2-p-aminophenyl-4-phenyl-5-imino-Δ^2-1,3,4-thiadiazole **(3g)**	H	NH_2	H	H
2-(p-amino phenyl)-4-(2´´, 4´´-dinitrophenyl)-5-imino-Δ^2-1,3,4-thiadiazole **(3h)**	H	NH_2	NO_2	NO_2

Table 3: Result of antinociceptive activity of 1,3,4-thiadiazole derivatives.

Compound	Mean reaction time [mean ± SEM] (sec)					
	Time interval (min)					
	0	10	20	30	40	50
3a	7.532 ± 0.217	8.59 ± 0.143	9.41 ± 0.859[c]	10.78 ± 0.825[a]	7.49 ± 0.614	8.39 ± 0.771
3b	8.680 ± 0.434	10.90 ± 0.048[a]	10.36 ± 0.234[b]	11.88 ± 0.519[a]	8.79 ± 0.524	6.36 ± 0.547
3c	5.780 ± 0.668	9.57± 0.415[b]	9.51 ± 0.255[c]	14.69 ± 0.356[a]	10.11± 0.524[c]	5.54 ± 0.387
3d	6.004 ± 0.374	9.32 ± 0.618[c]	10.22 ± 0.890[b]	11.76 ± 0.878[a]	9.50 ± 0.659	5.87 ± 0.629
3e	6.290 ± 0.928	9.46 ± 0.321[b]	9.94 ± 0.211[c]	11.14 ± 0.309[a]	8.15 ± 0.317	6.92 ± 0.206
3f	5.990 ± 0.283	8.80 ± 0.240	9.98 ± 0.264[b]	12.61 ± 0.432[a]	7.18 ± 0.668	5.76 ± 0.542
3g	4.830 ± 0.470	11.60 ± 0.762[a]	12.55± 0.378[a]	11.98 ± 1.39[a]	9.75 ± 0.239	7.45 ± 0.681
3h	6.460 ± 0.620	8.38 ± 0.713[b]	10.37 ± 1.07[b]	12.12 ± 0.377[a]	8.72 ± 0.69[c]	5.94 ± 0.807
Control	7.31 ± 0.226	6.87 ± 0.340	7.02± 0.457	6.41 ± 0.377	7.64 ± 0.198	7.18 ± 0.668
Standard	9.99 ± 0.269	11.18 ± 0.286	12.7 ± 0.134	14.85 ± 0.242	11.3 ± 0.271	10.7 ± 0.172

Notes: Standard drug is acetylsalycylic acid; control is distilled water, $p<0.001$ for a, $p<0.01$ for b, $p<0.05$ for c when compared with control. [SEM = standard error mean; formula = S.D/Vn (S.D = standard deviation; n = no. of animals)].

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