International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.1, No.4, pp 1200-1205, Oct-Dec 2009

2, 4- Di substituted-5-Imino-1, 3, 4- Thiadiazole Derivatives: Synthesis and Biological Evaluation of Anti-inflammatory activities

***MOHAMMAD ASIF[1], CHHAVI ASTHANA[2]**
[1]Department of Pharmacy, GRD (P.G) Institute of Management and Technology, Rajpur, Dehradun (U.K).248 009 INDIA.
[2]Department of Pharmacy, Bundelkhand University, Jhansi, UP, INDIA

****E-mail: mohd.mpharm@gmail.com Tel: +91-9897088910***

Abstract: The present work was to design, synthesize and investigate the in vivo anti-inflammatory activity of some 1, 3, 4-thiadiazole derivatives. 2, 4-diphenyl-5-imino-Δ^2-1, 3, 4-thiadiazole derivatives (IIIa-h) was prepared by Reaction of benzoyl chloride and phenyl hydrazine in pyridine yielded the corresponding substituted hydrazonoyl derivatives by condensation (Ia-h). Which were chlorinated to the corresponding α-chlorobenzal phenylhydrazone derivative (II-h) heating with phosphorous pentachloride and finally with cyclization of compounds (IIa-h) using potassium thiocyanate yields the targeted compound. The newly synthesized compounds were characterized by IR, ^{1}HNMR and elemental analysis. The titled compounds were screened for in vivo anti- inflammatory activity by carrageenan induced paw oedema method. Few of them manifested promising activity when compared to the standard drug Diclofenac sodium.

Key words: Antiinflammatory activity, oedema, hydrazonyl, thiadiazole.

Introduction

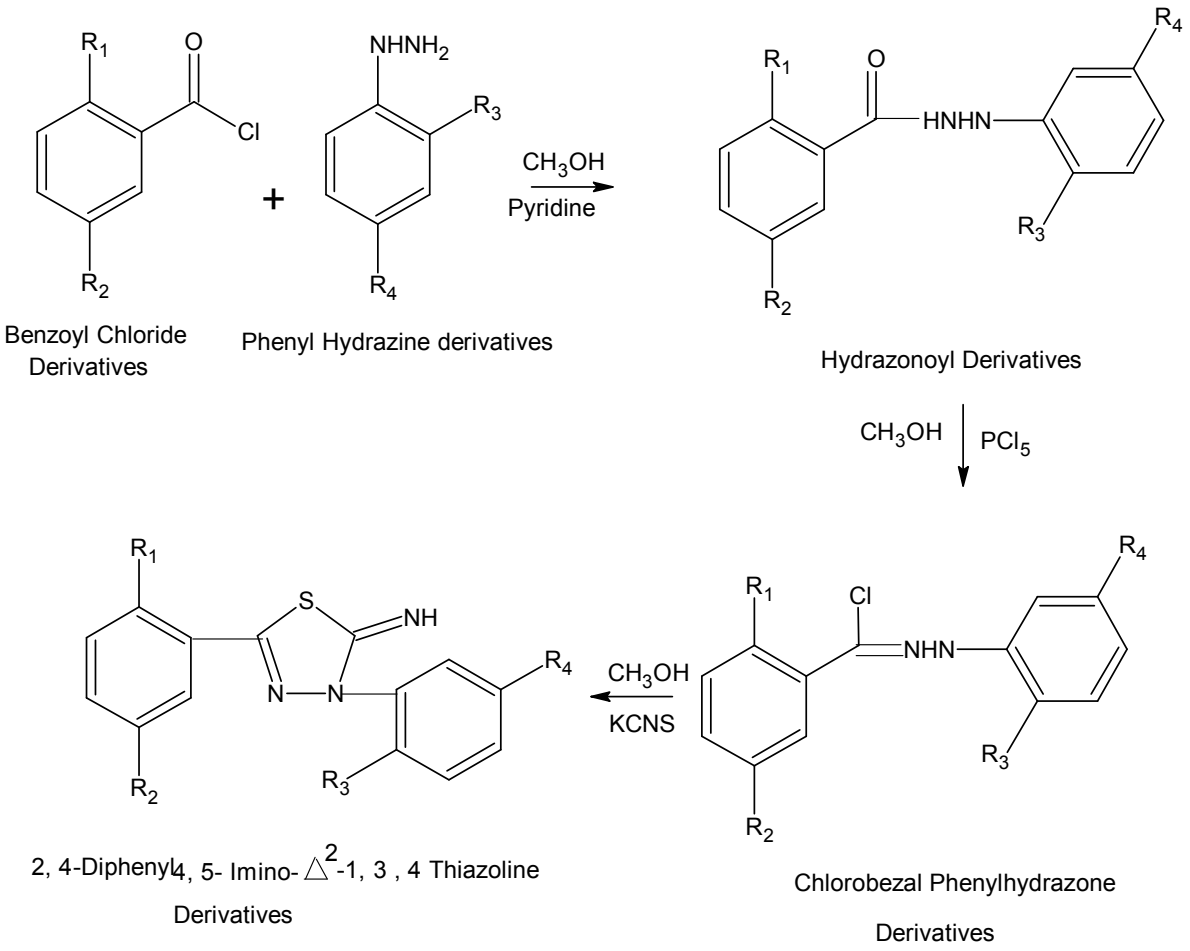
Most currently used nonsteroidal anti-inflammatory drugs (NSAIDs) have limitations for therapeutic use since they cause gastrointestinal and renal side effects that are inseparable from their pharmacological activities. Therefore, the synthesis of new compounds devoid of such side effects has become an important goal for medicinal chemists in recent years. For this purpose, various compounds incorporating a thiadiazole ring have been synthesized and their pharmacological activities have been reported[1-6].

In the family of heterocyclic compounds, nitrogen containing heterocycles with a sulpher atom are considered as an important class of compounds in medicinal chemistry because of their interesting diversified biological application. The synthesized compounds were evaluated for in vivo anti-inflammatory activity by carrageenan induced rat paw oedema method and the results are compared with standard drug Diclofenac sodium.

Chemistry & Spectral Characterization of the Compounds

The target compounds were synthesized as depicted in Scheme 1. The title compounds were synthesized as shown in reaction scheme by the following sequence of reactions. Substituted benzoyl chloride Condensation with phenyl hydrazine derivative in the presence of pyridine and methanol to obtained different substituted hydrazonoyl derivatives (1a-h).Chlorination of hydrazonoyl derivatives (Ia-h) yields substituted α-chlorobenzal phenylhydrazone derivative (IIa-h). Cyclization of substituted chlorobenzal phenyl hydrazone derivatives(IIa-h) with potassium thiocyanate in methanol yields the substituted 2,4-diphenyl-5-imino- Δ^2- 1,3,4-thiadiazole derivatives (IIIa-h).

The compounds were characterized on the basis of IR, ^{1}HNMR spectral data and elemental analysis. IR spectrum showed the characteristics bonds at 670, 1600 and 3400 cm^{-1} for -C-S-C-, -C=N-, and NH (imino) groups. The ^{1}HNMR spectrum showed the multiplet signal at 6.8-8.0, for aryl hydrogen's and singlet at 8.0-9.0 for amino NH at C-5 position of the ring. Presence of other substituents was also confirmed by IR as well as ^{1}HNMR spectral data. Elementary analyses were performed on a LECO CHNS 932 analyzer and satisfactory results of calculated values (C, H, N) were obtained. Physical characteristic data, elemental analysis and structure of (3a-h) compounds are given in table 1.



Scheme I

Materials and Methods

The identification of test compounds in the former stages of the experiment was done by the TLC method and the most common solvent system used were toluene, ethyl acetate, and formic acid (TEF) in the ratio of 5:4:1 and benzene and acetone (BA) in two ratios 5:1 and 4:1.Melting point 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.

General Procedure of Synthesis of Substituted Hydrazonoyl Derivatives (Ia-h) The first step of the synthesis was commenced with the reaction between (0.01M) benzoyl chloride or its derivatives were dissolved in methanol and ethanol (10 to 25ml) and then (0.005M) pyridine was added along with (0.01M) of phenyl hydrazine or its derivatives and then the reation mixture was refluxed at 50-60°C for 2 to 15 hours depends on derivatives used in the reaction mixture. The reaction time was monitored by TLC. Then we get different hydrazonoyl derivatives (Ia-h) as the product. These products were recrystallized by ethanol.

General Procedure of Synthesis of Substituted α-Chlorobenzal Phenylhydrazone Derivatives (IIa-h) The substituted hydrazonoyl and its derivatives (1a-h) (0.01-M) is chlorinated using (0.01M) PCl_5 in methanol and ethanol used vas solvent and then refluxed for 5- 12 hours at 40-60°C depend on the different derivatives used in this reaction.

The reaction time was monitered by TLC, different α -chlorobenzal Hydrazone derivatives (IIa-h) was obtained after keeping the mixture overnight. The recrystallization of these compounds from ethanol.

General Procedure of Synthesis of Substituted 2,4-Diphenyl-5-imino- Δ^2- 1, 3, 4- Thiadiazole Derivatives (IIIa-h): (Cyclization) Cyclization of substituted α-chlorobenzal phenylhydrazone derivatives(0.003M) with potassium thiocyanate (0.005M) in ethanol and methanol used as solvent. The reaction mixture was refluxed 50-70°C for 3-10 hours .The reaction time was monitored by TLC, and when the reaction completes the reaction mixture was kept overnight to get the crystals of products (IIIa-h).The recrystallization of these compounds from ethanol.

Experimental Animals:

Male Swiss albino rats (150 -200 g) were used. All the animals were left for 2 days in the laboratory for

acclimatization before the day of experiment, and on the last day they were given water only. Minimum of 5 animals were used in each group. All pharmacological activities were carried out as per CPCSEA (Committee for the purpose of control and supervision of experiments on animals) norms after obtaining the approval from the institutional animal ethical committee.

Anti-inflammatory Activity

Carrageenan-Induced Oedema Model

For the determination of the effects on acute inflammation, the carrageenan-induced paw edema model was employed7. Test samples and indomethacin used as a reference were administered orally respectively at doses of 100 mg/kg and 10 mg/kg as a suspension in 0.2 mL of 0.5% CMC Na. Sixty minutes after the oral administration of test samples and reference or dosing vehicle, the subplantar tissue of the right hind paw of each rat was injected with a freshly prepared 0.1 ml of 1% carrageenan suspension in physiological saline (154 mM NaCl). For the control, 10ml/kg saline solution was administered. Paw oedema was measured after 60 min, 90 min and 180 min after the induction of inflammation. Mean values of the treated groups were compared with those of the control group and analyzed using statistical methods (Table 2).

$$\text{Percentage Inhibition} = \frac{\text{Mean paw inflammation of control - Mean paw inflammation of test}}{\text{Mean paw inflammation of control}} \times 100$$

Table 1 Physico-chemical data for the synthesized compounds

Compd. No.	M.P °C	Mol. Formula	Mol. Wt	% Yield	Elemental Analysis Found/calcd. (%)	
3a	200	253	38	$C_{14}H_{11}N_3S$	H	4.37/4.31
					C	66.37/66.42
					N	16.58/16.49
					S	12.65/12.58
3b	202	343	75	$C_{14}H_9N_5O_4S$	H	2.64/2.61
					C	48.97/48.93
					N	20.39/20.38
					S	9.33/9.40
3c	198	287.5	57	$C_{14}H_{10}N_3SCl$	H	3.50/3.52
					C	58.43/58.37
					N	14.60/14.67
					S	11.14/11.21
3d	164	377.5	53	$C_{14}H_8O_4N_5SCl$	H	2.13/2.16
					C	44.51/44.43
					N	18.53/18.48
					S	8.48/8.52
3e	147	287.5	62	$C_{14}H_{10}N_3SCl$	H	3.50/3.45
					C	58.43/58.35
					N	14.60/14.54
					S	11.14/11.17
3f	210	377.5	66	$C_{14}H_8O_4N_5SCl$	H	2.13/2.15
					C	44.51/44.47
					N	18.53/18.57
					S	8.48/8.43
3g	238	268	61	$C_{14}H_{12}N_4S$	H	4.50/4.53
					C	62.662/62.61
					N	20.87/20.83
					S	11.94/11.87
3h	252	294	56	$C_{14}H_{10}N_6O_4S$	H	2.81/2.76
					C	46.92/46.84
					N	23.45/23.54
					S	8.94/8.98

Table 2 Anti-inflammatory activity of compounds (IIIa- h)

Compound. No.	Anti-inflammatory activity Inhibition of oedema %		
IIIa	60 min	90 min	180 min
IIIb	18.5	21.7	26.3 *c*
IIIc	15.8	17.8	20.4 *c*
IIId	13.6	15.9	20.6 *c*
IIIe	23.9	22.2	23.8*c*
IIIf	21.3	20.1	25.8*c*
IIIg	20.4	26.3	38.5*a*
IIIh	21.2	23.9	26.7*c*
Indomethacin	35.6 *c*	34.7*b*	40.1*a*

*a*P <0.001; *b*P <0.01; *c*P <0.05. *d*Anti-inflammatory activity of indomethacin was tested at 10 mg/kg dose.

Results and Discussion

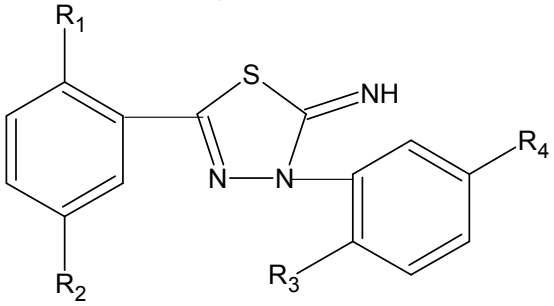
All 1, 3, 4- thiadiazole derivatives were synthesized and the structure of compounds were established by means of IR, ^{1}HNMR spectral data as well as elemental analysis. All the compounds were evaluated for anti-inflammatory activity. The compounds (IIIa- h) were obtained by the treatment of benzoyl chloride and phenyl hydrazine in pyridine yielded the
corresponding substituted hydrazonoyl derivatives (Ia-h). Which were chlorinated to the
corresponding α-chlorobenzal phenylhydrazone derivatives (II-h) heating with phosphorous pentachloride and finally with cyclization of compounds (IIa-h) using potassium thiocyanate yields different thiadiazole derivatives (Table 3). The yield was found to be in the range of 38-74%.

The titled compounds were confirmed by IR spectral data showing characteristic bands at 3400-3500 cm-1 indicated the presence of –NH stretching, sharp bands in the range between 1601-1661 cm-1 indicated the presence of C=N group. Compounds (IIIb, IIId, IIIf and IIIh) were confirmed by stretching at 1340 cm-1 due to the presence of -NO2 group. Similarly compounds (IIIc-IIIf) were further confirmed by a sharp absorption peak at 769 cm-1 denoted the presences of C-Cl group.Compounds (IIIa-h) were confirmed by 1HNMR spectralanalysis. The NMR proton multiplet peak at 7.2-8.2 ppm and 3.73 ppm revealed the presence of aryl and NH2 groups (Table 4). The synthesized compounds (IIIa-f) with functional groups such as Cl, NO_2, were found to have exhibited less anti-inflammatory activity, whereas compound IIIg i.e. 2-p-aminophenyl-4-phenyl-5-imino-Δ^2-1, 3, 4-thiadiazole was found to possess better activity with 35.5 % inhibition.

Conclusions

The yield of all 2, 4-Disubstituted-4 imino-1, 3, 4-thiadiazole derivatives were found to be in the range of 38-74%. The purity of compounds were ascertained by melting point and TLC. The assigned structure was further established by IR, 1HNMR and elemental analysis studies. The acute anti-inflammatory activity of the synthesized compounds was screened using the carrageenan induced paw oedema method in rats. Diclofenac sodium was used as a reference drug. In the prepared thiadiazole series it seemed that compound IIIa-IIIf and IIIh showed P <0.05, when compared to the standard drug Diclofenac sodium. But the compound IIIg, 2-p-aminophenyl-4-phenyl-5-imino-Δ^2-1, 3, 4-thiadiazole exhibited highest anti-inflammatory activity (P <0.0001) with a percentage inhibition of 35.5. From the present study, it may be concluded that the thiadiazole compounds can potentially be developed into useful anti-inflammatory agents that can prompt future researchers to synthesize a series of thiadiazole derivatives containing a wide variety of substituents with the aim of obtaining novel heterocyclic systems with enhanced activity. Further work to develop and improve similar and related compounds and test them for manifold biological activity is in progress.

Table 3 Structure of different synthesized 1, 3, 4 - thiadiazole derivatives and their elemental analysis



2, 4-Diphenyl4, 5- Imino- $\triangle^2$-1, 3 , 4 ThiazolineDerivatives

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 (5d)	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 4 IR $cm^{-1}$and ^{1}H NMR Spectral Data of Compounds (IIIa-h)

IR cm^{-1}

IR cm^{-1} 1397(Ar- NH_2), 1631(C=N), 3410(NH), 686(C-Cl), **IR cm^{-1}** 1349 (NO_2), 1594(C=N), 3442 (NH). **IR cm^{-1}** 692(C-S-C), 1601 (C=N), 3391(NH)**IR cm^{-1}** 1519 (NO_2), 661 (C-S-C), 1661(C=N), 1598 (C=N,imine), 3374(NH) **IR cm^{-1}** 670 (C-S-C), 1595(C=N), 1500(NO_2), 3440(NH). **IR cm^{-1}** 672(C-S-C), 1524 (C=N), 1340(NO_2), 3450(NH). **IR cm^{-1}** 672 (C-S-C),1351(NO_2), 769 (Ar- Cl),1595, (C=N),3433(NH).**IR cm^{-1}** 1398 (Ar-NH), 1631(C=N), 692(C-S-C), 3442(NH). **IR cm^{-1}** 1350(NO_2), 1595(C=N), 671(C-S-C), 3443(NH).

^{1}H NMR (DMSO-d_6) ppm

^{1}H NMR 6.8-7.34 (10H, m, Ar-H), 8.32(1H, s, NH imine). **^{1}H NMR** 7.3-8.2 (5H, m, Ar), 10.9 (1H, s, NH), 8.3-9.14 (3H, m, Ar-NO_2). **^{1}H NMR** 7.2-7.9 (9H, m, Ar) 3.9(1H, s, NH). **^{1}H NMR** 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). **^{1}H NMR** 7.1-7.8(4H,m,Ar-Cl),8.2-8.9(3H,m,ArNO_2),8.58 (1H,s,NHimine)**^{1}H NMR** 8.2-9.0 (4H, m, Ar-Cl), 9.3 (1H, s, NH), 7.2-7.8 (3H, m, Ar- NO_2). **^{1}H NMR** 7.2-8.0(9H, m, Ar), 3.7(1H, s, NH).

Acknowledgement

The authors are thankful to Bundelkhand University, Jhansi, GRD (PG) Institute of Management & Technology, Dehradun, India as well as to SAIF, Punjab University, Chandigarh, and CDRI Lucknow, India for providing technical support and facilities to carry out this work.

References

1. Adnan A.K, Nasser R.E, Omar A.A, Elsayed E.H, Tarek M.I, ALI A.E. Synthesis, antimicrobial, and anti-inflammatory activities of novel2-(1-adamantyl)-5-substituted-1, 3, 4-oxadiazoles and 2-(1-adamantylamino)-5-substituted-1, 3, 4-thiadiazoles, Eur J Med Chem, 2007, 42 235-242.
2. Amir M, Kumar H, Javed SA (2007)Synthesis and pharmacological evaluation of condensed heterocyclic 6-substituted-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole derivatives of naproxen. Bioorganic & Medicinal Chemistry Letters. 15, 17(16):4504-8.
3. Rajesh S, Jitendra S, Subhash C C. (2008) 2-Amino-5-sulfanyl-1, 3, 4-thiadiazoles: A new series of selective cyclooxygenase-2 inhibitors *Acta Pharm. 58* 317–326
4. Song Y, Connor DT, Sercel AD, Sorenson RJ, Doubleday R, Unangst PC, Roth BD, Beylin VG, Gilbertsen RB, Chan K, Schrier DJ, Guglietta A, Bornemeier DA, Dyer RD. (2008) Synthesis, structure-activity relationships, and in vivo evaluations of substituted di-tert-butylphenols as a novel class of potent, selective, and orally active cyclooxygenase-2 inhibitors. 2. 1, 3, 4- and 1, 2, 4-thiadiazole series. European Journal Medicinal chemistry, 43(10):2056-66.
5. Thomas, E. W. In Comprehensive Heterocyclic Chemistry II; Katritzky, A. R.; Rees, C. W. Eds; Elsevier: Oxford, 1996; Vol. 4, p 447.
6. Thomas, E. W. In Comprehensive Heterocyclic Chemistry II; Katritzky, A. R.; Rees, C. W. Eds.; Elsevier: Oxford, 1996; Vol. 4, p 289.
7. Winter, C.A., Risley, E.A., Nuss, G.W. (1962) Carrageenan induced edema in hind paw of the rats as an assay of anti-inflammatory drug. Proc. Soc. Exp. Biol. Med., 111: 544 – 547.

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