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Synthesis, characterization of some new [1,3, 4]-oxadiazole derivatives as anti-inflammatory interest

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ABSTRACT

This Present investigation deals with the design, synthesis, spectral and biological activities studies of some new [l,3,4]-oxadiazole derivatives. The acid hydrazides derived from 2-(4-isobutylphenyl)propanoic acid and 4-methylthiophenyl acetic acid were subjected to cyclization with carbon disulphide under basic conditions to yield [l,3,4]-oxadiazol-2-thiones which on aminomethylation with formaldehyde and primary aromatic/secondary amines afforded a series of Mannich bases. Purity of the compounds has been confirmed by TLC. The structures of these newly synthesized compounds were established on the basis of their IR, ^{1}H NMR, ^{13}C NMR, 2D NMR and Mass spectral data. All the title compounds have been screened for their antimicrobial and for anti-inflammatory activities.

Keyword: Anti-inflammatory activity, 4-methylthiophenyl acetic acid

INTRODUCTION

Inflammation (latin, *inflammare,* to set on fire) is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammation is caused by release of chemicals from tissues and migrating cells. Most strongly implicated are the prostaglandins (pgs), leukotrienes (lts), histamine, bradykinin, and, more recently, platelet-activating factor (paf) and interleukin-1. Evidence for their involvement comes from studies with competitive antagonists for their receptors and inhibitors of their synthesis. Some of the anti-inflammatory drugs especially corticosteroids prevent the formation of both pgs and lts by causing the release of lipocortin, which by inhibition of phospholipase a2 reduces arachidonic acid release. They suppress the inflammation of rheumatoid arthritis and asthma. Unfortunately, corticosteroids are also associated with serious side effects such as high blood sugar, menstrual irregularities, easy bruising, thin skin, cataracts, increased risk of infections, reduced adrenal gland hormone production and loss of calcium from the bones.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are frequently used to treat it ii inflammatory conditions such as arthritis, bursitis and tendonitis[1,2]. NSAIDs are relatively inexpensive and are frequently the first line of medication used to relieve pain and reduce inflammation. Most NSAIDs act as non selective inhibitors of the enzyme cyclooxygenase (COX), inhibiting both the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isoenzymes[3-6]. COX catalyzes the formation of prostaglandins and thromboxane from arachidonic acid (itself derived from the cellular phospholipid bilayer by phospholipase A2). Prostaglandins act (among other things) as messenger molecules in the process of inflammation.

Through years of ingenious syntheses and structural modifications that usually follow the design and development of new drugs, many non-steroidal anti-inflammatory agents (NSAIDS) have been prepared and marketed. However, these drugs are known to provoke adverse effects such as gastrointestinal irritations. Hence, the search for novel anti-inflammatory drug is gaining

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importance in the recent years. Some evidences suggest that the oxadiazole moiety present in some compounds possess ananti-inflammatory activity by virtue of dual mechanism i.e., inhibiting both COX/Losto reduce gastric acid formation. The reported literature confirms that gastrointestinal side effects of arylpropionic acids are due to the presence of a free carboxylic groupin the parent drug[7-14]. Thus, developing new agents with minimum or without side effects is an extensive research area at present. The replacement of the terminal carboxylic function of propionic acid by oxadiazole ring may enhance the anti-inflammatory activity of such compounds with reduced ulcerogenic effects[15].

1, 3, 4-oxadiazole derivatives are heterocyclic compounds containing one oxygen and two nitrogen atoms in a five-membered-ring. 1,3,4-oxadiazole derivatives have played a major role in the pharmaceutical chemistry. The number of so many synthetic compounds with oxadiazole nucleus used for antibacterial[16-20], antifungal[21-24], analgesic and anti-inflammatory activities[25-28]. Derivatives of 1,3,4-oxadiazole with suitable substitution at 2,5-position have already been reported to have possible biological activities. 1,3,4-oxadiazole derivatives act as anticonvulsant and diuretics[29]. These observations and our interest in the pharmaceutical chemistry of heterocyclic compounds promoted us to have synthesized different derivatives of 1,3,4-oxadiazole with different substituent at 2 and 5-positions. These derivatives have been also screened for their anti-inflammatory activity.

Mostly, five-membered-ring aromatic systems having three heteroatoms at symmetrical position have been studied because of their physiological properties[30,31]. It is also well established that various derivatives of 1,3,4-oxadiazole exhibit broad spectrum of pharmacological properties such as antibacterial and antifungal activities[32,33]. 1,3,4-oxadiazole showed antibacterial properties similar to those of well known sulphonamide drugs[34].

Materials and methods

Experimental

Ibuprofen [2-(4-isobutylphenyl) propanoic acid] and 4-(Methylthiophenyl)acetic acid were obtained commercially and used as such without further purification. The melting points were determined by an open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT IR 157 spectrophotometer. The ^{1}H NMR, and ^{13}C NMR spectra were recorded (CDC b /DMSO-ds mixture) on a BRUKER AVANCE II -400 (400 MHz) spectrometer using TMS as an internal standard. Mass spectra were recorded in Agilent Technology LC-mass spectrometer and MS spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer using argon/xenon (6kv, 10mA). Elemental analyses (CHNS) were performed on the CHNS Elementar Vario EL III. The progress of the reaction was monitored by thin layer chromatography (TLC) on silica gel plates.

Current working Scheme

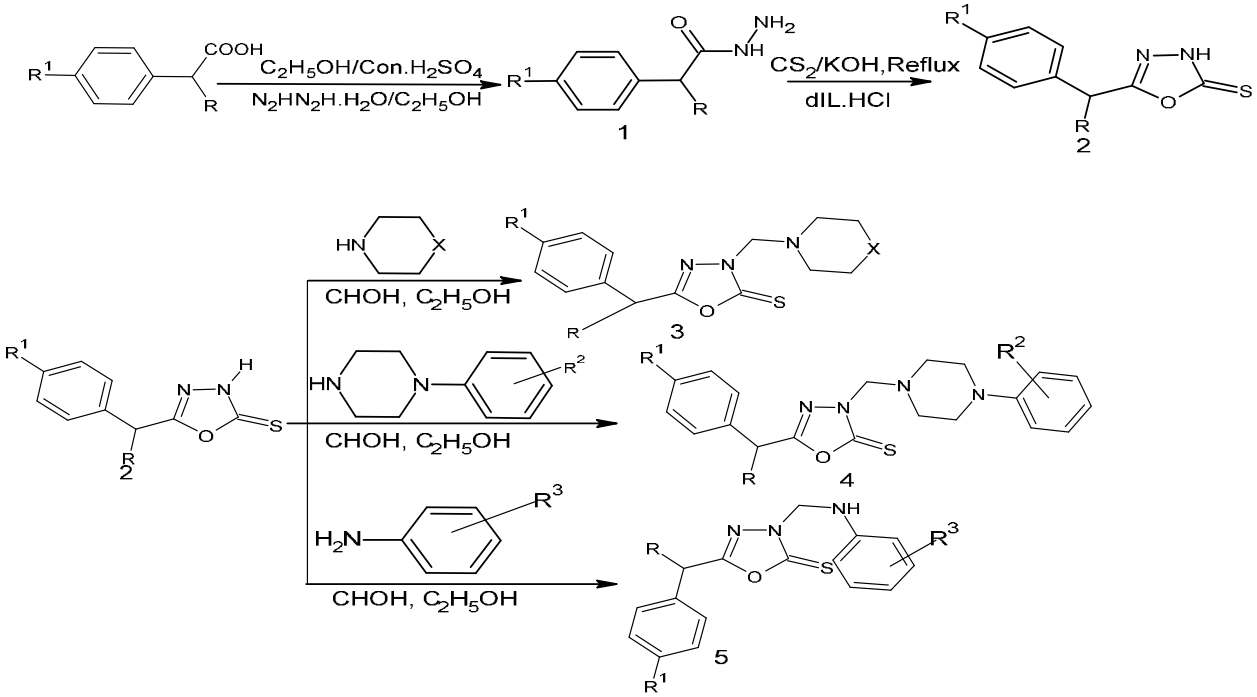

Figure – 1: **Synthesis Scheme for 5-substituted-[l,3,4]-oxadiazole-2(3//)-thiones**

Compound	R	R1	R2	R3	X
3	H	SCH_3	-	-	O
4	H	SCH_3	4-NO_2	-	-
5	H	SCH_3	-	2,4-Cl	---

General Procedure for the Preparation of Arylhydrazines (1)

The ethyl esters were prepared by refluxing substituted aromatic acids in excess absolute ethanol in the presence of few drops of cone, sulfuric acid as per the general method employed for the esterification61. The resulting esters had been judged to be pure by TLC.The mixture of ethyl ester of substituted aromatic acids (0.1 mol) and hydrazine hydrate (0.2 mol) was refluxed in absolute alcohol (50 mL) for 8 h. The excess solvent was then distilled off under reduced pressure and the concentrated solution was quenched in to ice cold water. The solid separated was filtered, washed and dried. The crude product was purified by recrystallization from ethanol.

General Procedure for the Preparation of 5-Aryl-2-mercapto-[l,3,4]-oxadiazole (2)

A mixture of aroyl hydarzide **(34)** (0.1 mol), KOH (5.6 g, 0.1 mol) in absolute alcohol (50 mL) and CS2 (15.2 g, 0.2 mol) was taken in a round bottom flask and refluxed for about 4 h till the evolution of hydrogen sulfide was ceased. The reaction mixture was cooled to room temperature and diluted with water. The product precipitated out on acidification with dilute hydrochloric acid was filtered, thoroughly washed with cold water and recrystallized from ethanol.

General Procedure for the Preparation of Mannich Bases (3, 4 and 5)

To a solution of 5-aryl-2-mercapto-[l,3,4]-oxadiazole-2-thione **35**(10 mmol) in ethanol (15 mL), a mixture of formaldehyde (0.45 g, 15 mmol) and a secondary amine (10 mmol) in 10 mL ethanol was added with stirring. After complete addition, the stirring was continued overnight at room temperature. The precipitated solids were filtered, washed with water and dried. The crude product was recrystallized from ethanol.

Anti-inflammatory activity[35]

Anti-inflammatory activity of all synthesized derivatives was determined by the carrageenan-induced rat paw oedema model. Albino rats (100-200 g) were divided into 5 groups (six animals per group). Overnight fasted animals were used and during that period only tap water was given.Group I received 0.6 % Na CMC (sodium carboxy methyl cellulose) and the Group II received Diclofenac Sodium at a dose of 10 mg/kg body weight *p. o.* The Group III to Group V groups were administered with the test compounds at a dose 10 mg/kg (suspended in 0.6 % CMC given *p. o.).* Thirty minutes after the treatment of test compounds, 0.1 ml of 1 % (w/v) carrageenan was injected in the subplantar region of the left hind paw. The right paw served as a reference to non-inflamed paw for comparison. The initial paw volume was measured within 30 s of the injection. The relative increase in paw volume was measured in control, standard and test compounds at 3 h after the carregeenan injection. The difference between the two readings was taken as the volume of oedema & the percentage inhibition by the drugs was calculated using the formula,

$$\text{Percentage inhibition} = 100 - [\frac{Vtest}{Vcontrol} \times 100]$$

Where,
Vcontroi = volume of paw oedema in control group;
Vtest = volume of paw oedema in the test compounds in treated group.
The results were expressed as % inhibition of oedema over the untreated controlgroup. The results of anti-inflammatory studies are given in Table 2.

RESULT AND DISCUSSION

At the end of the experiment, it has been concluded that the compounds synthesized have good yield value. The synthesized 1,3,4-oxadiazole compounds were identified and characterized by IR, ^{1}H NMR, ^{13} C NMR and MASS spectra. Then, the pharmacological activity was done. The entire compound had a good response for Anti-inflammatory activity: 5-(4-Methylthiobenzyl)-3-[(4-methylpiperazin-l-ylmethyl)-[l,3,4]-oxadiaz0le-2(3H)- thione (3), 5-(4-methylthiobenzyl)-3-{[4-(4-nitrophenyl)piperazin-l-yljmethyl}-[13,4J-oxadiazoie-2(3H)-thione (4) and 5-(4-methylthiobenzyl)-3-{[4-(2,4-dichlorophenyl)]methyl}-[l,3,4]-oxadiazole-2(3H)-thione (5).

Table 1: Physical Characterization data of 5-substituted-[l,3,4]-oxadiazole Mannich bases 3,4 and 5.

Compound	Mol. Formula	Mol. Wt.	M.P ºC	% yield
3	$C_{15}H_{19}N_3O_2S_2$	337	88–90	62
4	$C_{21}H_{23}N_5O_3S_2$	457	168-170	82
5	$C_{18}H_{17}N_3OSCl_2$	393	140-142	85

Table 2: Anti-inflammatory activity data of 5-substituted-[l,3,4]-oxadiazole

Compound	Dose (mg/kg body weight,*p.o)*	Increase in paw volume in ml (MEAN ± SEM)	% Inhibition of paw oedema
3	10	0.362 ± 0.0025	35.17
4	10	0.242 ±0.0021	56.61
5	10	0.175 ± 0.0034	68.71

Control	0.1 ml/kg	0.156 ±0.031	----------
Standard	10	0.018 ± 0.0027	73.66

Result was Mean ± SD, n = 6

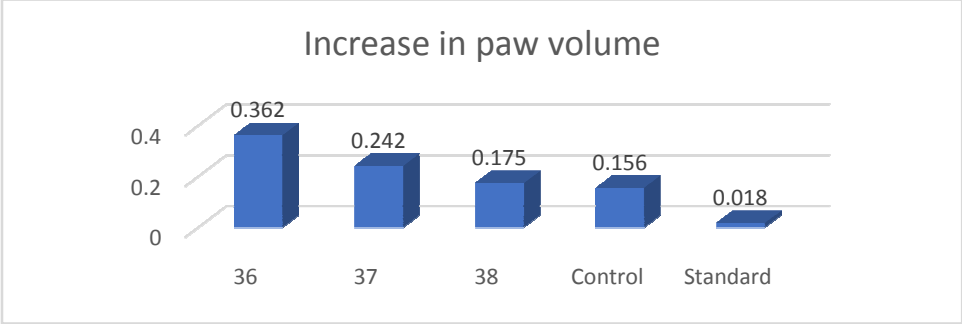

Figure 2:Anti-inflammatory activity data of 5-substituted-[l,3,4]-oxadiazole

The IR spectrum of Mannich base **3** showed the absence of absorption bands corresponding to the NH group of the parent oxadiazole. It showed absorption bands at 3085 for aromatic C-H, 1617 cm-1 for C=N, 1323 cm-1 for C=S and 1246 cm-1 for C-O stretching vibrations. The 400 MHz ^{1}H NMR spectrum 36a showed the signals corresponding to the NH/SH tautomeric proton was absent and a new singlet for N-CH_2-N was observed at £4.89, thus confirming the aminomethylation. It also showed prominent singlets at δ 2.46 and δ 4.13 for its SCH_3 and CH_2 protons respectively. Two characteristic triplets at δ 2.65 and 8 3.54 each integrating for four protons were due to the methylene protons of the morpholine ring. The four protons of 4-methylthiophenyl moiety appeared as multiplet in the range δ 7.24-7.29. The 400 MHz ^{13}C NMR spectrum of compound 36a showed characteristic signals at δ 14.62, 30.34, 49.93, 65.93, 69.52, 126.22, 129.58, 137.42, 161.07 and 177.79. The FAB mass spectrum of 36a showed a protonated molecular ion (M++l) peak at m/z 337 along with the molecular ion (M+) peak at m/z 336, consistent with its molecular formula $C_{15}H1_9N_3O_2S_2$. The base peak was observed at m/z 100 due to the formation of morpholinomethyl cation.

The structures of hydrazides **(1),** 5-substituted-[l,3,4]-oxadiazol-2(3//)-thiones **(2)** and their Mannich derivatives **(3, 4**and **5)** were established on the basis of elemental analyses, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data.Characterization data of all the newly synthesized compounds are presented in Table1.

Formations of the Mannich bases **(3, 4**and **5)** were also confirmed by their element alanalyses, IR, ^{1}H NMR, ,^{13}C NMR, and Mass spectral data. The IR spectrum of Mannich base **3**showed the absence of absorption bands corresponding to the NH group of the parent oxadiazole. It showed absorption bands at 3085 for aromatic C-H, 1617 cm-1 for C=N, 1323 cm-1 for C=S and 1246 cm-1 for C-O stretching vibrations. The 400 MHz ^{1}H NMR spectrum **3** showed the signals corresponding to the NH/SH tautomeric proton was absent and a new singlet for N-CH_2-N was observed at δ 4.89, thus confirming the aminomethylation. It also showed prominent singlets at δ 2.46 and £4.13 for its SCH_3 and CH_2 protons respectively. Two characteristic triplets at δ 2.65 and 8 3.54 each integrating for four protons were due to the methylene protons of the morpholine ring. The four protons of 4-methylthiophenyl moiety appeared as multiplet in the range δ 7.24-7.29.

The 400 MHz ^{13}C NMR spectrum of compound **3** showed characteristic signalsat δ 14.62, 30.34, 49.93, 65.93, 69.52, 126.22, 129.58, 137.42, 161.07 and 177.79.The mass spectrum of **3** showed a protonated molecular ion (M++l) peak at ***m/z*** 337 along with the molecularion (M+) peak at ***m/z*** 336, consistent with its molecular formula $C_{15}H_{19}N_3O_2S_2$. Thebase peak was observed at m/z 100 due to the formation of morpholinomethyl cation.

The IR spectrum of the Mannich base **4**showed characteristicabsorption bands at 3062 cm^{-1}for aromatic C-H, 2849-2954 cm^{-1}for aliphatic/alicyclic C-H, 1593 cm^{-1}for C=N and 1317 cm^{-1}for C=S groups. The asymmetric and symmetric stretching vibrations of NO_2group are observed at 1445 and 1113 cm^{-1}respectively.The 400 MHz 'H NMR spectrum of **4** showed a characteristic singlet at *δ* 2.43 integrating for three protons of the SCH_3 protons. Two triplets at *δ 2.79* and *8* 3.46 each integrating for four protons were due to the methylene protons of the piperazine ring. Two singlets at δ4.10 and δ 4.98 each integrating for two protons were due to the CH_2 and N-CH_2-N protons, respectively. The four protons of the 4-methylthiophenyl ring resonated as a multiplet in the region *δ*7.19-7.25. The four aromatic protons of the 4- nitrophenylpiperazine ring appeared as two doublets centred at *δ* 6.99 and 8.02 with *J*= 9.6 Hz. The mass spectrum of **4** showed a protonated molecular ion (M++l) peak at *m/z* 458 consistent with its molecular formula $C_{21}H_{23}N_5O_3S_2$.

The IR spectrum of compound **5**spectrum showed a broad absorption band at 3076 cm^{-1} for its N-H moiety. The other characteristic absorption bands at 1611 cm^{-1} for C=N, 1326 cm $^{-1}$ for C=S and 695 cm $^{-1}$, 721cm $^{-1}$ for C-Cl groups, were also observed in the spectrum. The 400 MHz !H NMR spectrum of Mannieh base **5**showed triplet centered at δ 4.69 integrating for one proton of NH group with *J* = 5.6 Hz. The methylene proton appeared as a doublet at δ 5.45 with a coupling constant *J* = 5.6 Hz. Two singlets at δ 2.46 and δ 4.11 integrating for three and two protons were due to the SCH_3 and CH_2 groups, respectively. The four protons of the 4-methylthiophenyl ring appeared as multiplet in the range δ 7.20-7.25. The three protons of the 2,4-dichlorophenyl ring

appeared a triplet (overlapped doublet of doublets) and two doublets centered at δ 6.87 (C_3H), 7.07 (J = 8.8 Hz, C_5H) and 7.44 (J = 2.4 Hz, CSH), respectively. The 400 MHz ^{13}C NMR spectrum of
compound **5**showed characteristic signals at δ 14.59, 30.35, 56.30, 111.56, 112.38, 125.81, 125.93, 126.16, 129.39, 129.66, 136.75, 137.48, 138.09, 151.98, 153.17, 161.80 and 176.06. The mass spectrum of this compound showed a protonated molecular ion (M^++l) peak at ***m/z*** 412 along with the molecular ion (M^+) peak at ***m/z*** 411 & M+2 peak at m/z = 413, consistent with its molecular formula $C_{17}H_{25}C_{l2}N_3OS_2$.The anti-inflammatory activity of oxadiazole Mannich bases derived from 4-thiomethylphenyl acetic acid was in the range of 35.17 % to 68.71 % inhibition. The highest activity (68.71 %) was found for the Mannich base 5.

CONCLUSION

Various [l,3,4]-oxadiazole Mannich bases derived from ibuprofen and 4- methylthiophenyl acetic acid were prepared with the objective of developing better anti-inflammatory agents andto evaluate their antimicrobial potency. It was interesting to note that three compounds 36, 37and 38were found to have anti-inflammatory activity with compared to standard drug, diclofenac at 10 mg/kg *p.o.* and 38 showed maximum activity. Further study is required to know the mechanism of action of the synthesized compound for anti-inflammatory activityand thus emerged out as potential lead for the development of novel anti-inflammatory agent with good efficacy.

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