

Pharmacological Evaluation of 5-Aryl-1,3,4-Oxadiazole-2(3h) Thione Derivatives as Anti-Inflammatory Agents

Shailesh Pathak¹, Sharad Sharma^{2*}, Ram Ratan², Khushboo Lavania³ and Mahesh Prasad¹

¹Kamla Nehru Institute of Management and Technology, Sultanpur, Uttar Pradesh Pin code: 228118, India

²School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, Pin code: 302017, India

³Anand College of Pharmacy, Keetham, Agra, Uttar Pradesh Pin code: 282007, India

ABSTRACT

The Latin word 'inflammation' means to set on fire. Vasodilation which increases blood flow is one of main events which results the skin redness, and other event is swelling because of interstitial fluid increases as capillaries and venules become leakier. Anti-inflammatory drugs are used to cure inflammation. The pharmacological activity of NSAIDs is due to suppression of prostaglandin biosynthesis from arachidonic acid by inhibiting the enzyme cyclooxygenases (COXs) which exists in two isoforms, COX-1 and COX-2, which were regulated differently. 1,3,4-oxadiazoles represent a class of heterocyclic compounds of great importance in biological chemistry.

Keywords: Inflammation, Vasodilation, Defensive mechanism, Pharmacological screening

INTRODUCTION

Inflammation is a multi component response to tissue stress, injury and infection, and a crucial point of its control is at the level of gene transcription [1]. Inflammation is a local protective response of body to the tissue injury. It is a defensive mechanism to any noxious stimulus; which may vary from a localized to a generalized response characterized by the accumulation of fluids and leukocytes resulting edema and pain [2]. At the time of acute inflammatory responses, cellular and molecular interactions efficiently minimize impending injury or infection [3]. Factors like infection, tissue injury can enhance inflammation which results tissue damage. The body gives chemical signaling in tissue injury which stimulates responses in healing [4]. Inflammation is defined as a tissue directed response to noxious external and internal stimuli, which is predominantly mediated by arachidonic acid metabolites. The inflammatory response has several beneficial effects:

*Corresponding Author

me.sharadsharma[at]rediffdotcom

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- Prevents the spread of damaging agents to nearby tissues
- Disposes cell debris and pathogens
- Sets the stage for repair [5]

Type of Inflammation

The inflammatory responses playing an important role in acute and chronic inflammation (Figure 1). In acute inflammation there is initial response to the tissue injury, And chronic inflammatory involves the release of diverse mediators like interleukins, interferon, cytokine etc [6,7].

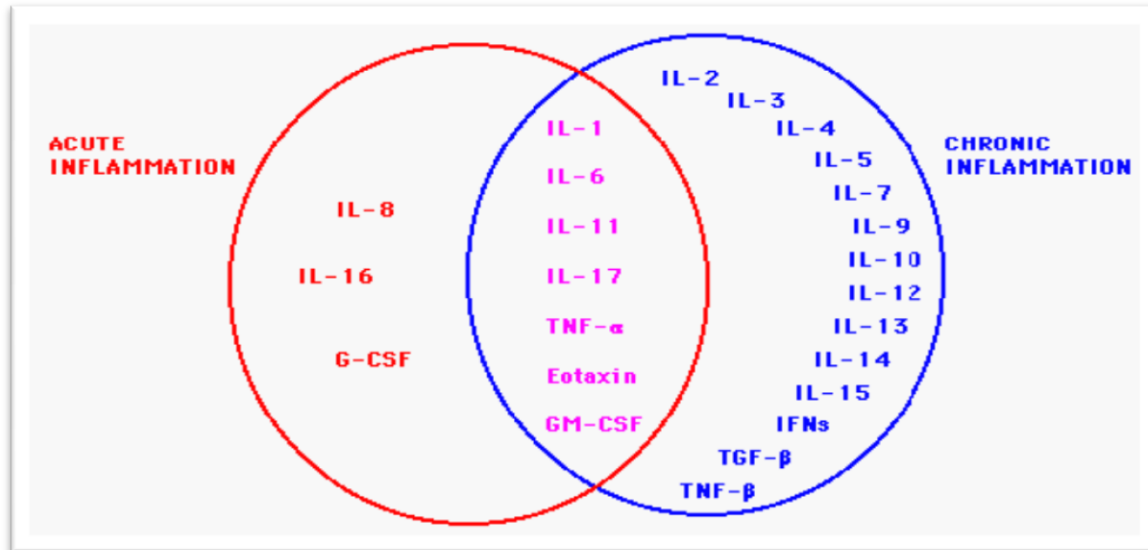


Figure 1: Acute and Chronic inflammation

Acute Inflammation

Acute inflammation is of short duration and represents the early body reaction and is usually followed by repair

Causative Stimuli

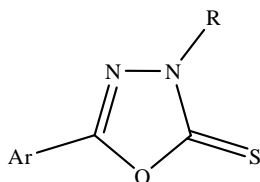
1. Infections (bacterial, viral, parasitic) & microbial toxins
2. Trauma (blunt & penetrating)
3. Physical (burns, frostbite, irradiation) & chemical agents
4. Tissue necrosis (from any cause)
5. Foreign bodies (splinters, dirt, sutures)
6. Immune reactions (hypersensitivity reactions)

Major Manifestations

1. Rubor (redness) due to vasodilatation- the widening of blood vessels
2. Tumor (swelling) due to an influx of fluid into the damaged region
3. Calor (heat) due to an increase in blood flow to the area
4. Dolor (pain) due to chemicals released by damaged cells
5. Functio laesa(loss of function) due to increased swelling and pain [8]

1,3,4-OXADIAZOLES

Nucleus

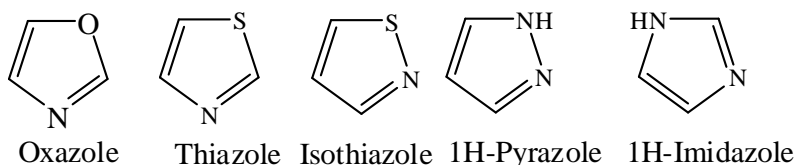


5-aryl-1,3,4-oxadiazole-2(3H)thione derivatives

Oxadiazole ring possesses anti-inflammatory properties in compounds designed as orally-active nonulcerogenic agents. The inhibition of monoamine oxidase involved a series of tricyclics bearing oxadiazole moieties in structure–activity relationship study [9].

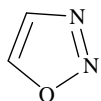
Chemistry

The group of five-membered aromatic heterocycles is much larger than that of the six-membered heterocycles. This is because one of the atoms in the ring need only be divalent and so more heteroatoms can be incorporated into neutral five-membered rings [10].

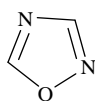


Oxadiazole

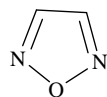
Oxadiazoles are considered to be derived from furan by replacement of two methine (-CH=) groups by two pyridine type nitrogens (-N=). The hetero aromatic oxadiazole ring contains two carbon atoms, two nitrogen atoms and one oxygen atom, which can be arranged in four combinations to give either 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole or 1,3,4-oxadiazole.



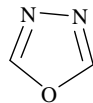
1,2,3-oxadiazole
Furo [ab] diazole
(Diazoxide)



1,2,4-oxadiazole
Furo [ab1] diazole
(Azoxime)



1,2,5-oxadiazole
Furo [aa1] diazole
Azoxazole
(Furazan)



1,3,4-oxadiazole
Furo [bb1] diazole
Oxybiazole
(Oxadiazole)

Properties of Oxadiazole

Molecular formula : C₂H₂N₂O

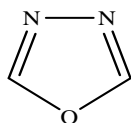
Molecular weight : 70.05

Physical state : liquid

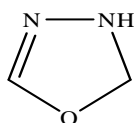
Boiling point : 150 °C

Structure

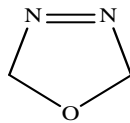
1,3,4 oxadiazole (1) is a thermally stable neutral aromatic molecule, only the isomer not containing oxygen-nitrogen bond and exists in two partially reduced form; 2,3 dihydro- 1,3,4- oxadiazole (2) and 2,5 dihydro 1,3,4- oxadiazole (3) depending on position of double bond. The completely reduced form of 1,3,4- oxadiazole is designated as 2,3,4, 5- tetrahydro- 1,3,4- oxadiazole (4).



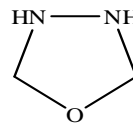
(1)



(2)



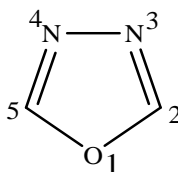
(3)



(4)

1,3,4-oxadiazole ring is symmetrical and planer with the following structural parameters.

Bond Length (Å) Bond angle (°)



[1,3,4]Oxadiazole

- N₃-N₄ = 1.399
- C₂-O-C₅ = 102.0
- C₂-N₃ = 1.297
- O-C₂-N = 113.4
- N₄-C₅ = 1.297
- C₂-N₃-N₄ = 105.6
- O-C₂ = 1.348
- N₃-N₄-N₅ = 105.6
- O-C₅ = 1.348
- O-C₅-C₄ = 113.4

1,3,4 – Oxadiazole is an aromatic, molecule with resonance energy 167.4kJ/mol. The bond lengths in the 1,3, –Oxadiazole reflect π - electron delocalization. However the C=N bond lengths are very close to that in acyclic compound(1.27Å) and therefore indicate some dienic character in 1,3,4-oxadiazole.

Inflammatory Mediators

Prostaglandin

Production of prostaglandins throughout the gastrointestinal tract and major mediators for a complex array of pathophysiological and physiological processes in the intestine. Intestinal myofibroblasts, which express COX and generate PGE₂, play important roles in intestinal epithelial proliferation, differentiation, inflammation, and neoplasia through secreting growth factors and cytokines [11].

Cytokines

Cytokines are huge hydrophilic molecules thus penetration through the B.B.B. is thought to be negligible and their role in pathology of psychiatric disorders limited. Cytokines may enter the brain, however, through area where the B.B.B. is absent (e.g., circumventricular organs), less restrictive (e.g. median eminence), compromised (e.g., multiple sclerosis) [12].

Nitric oxide & histamine

Nitric oxide is an important signal transducing molecule of various aberrant responses during systemic inflammation, including reduced organ functioning, reduced xenobiosis, and decreased drug action [13]. Histamine is a mediator which play important role in inflammatory mediator and can affect the function of monocytes, dendritic cells and lymphocytes [14,15].

Leukotrienes

LTs (leukotrienes) are inflammatory mediators derived from arachidonate by the 5-lipoxygenase pathway, which has been implicated in the pathogenesis of atherosclerosis. In this pathway, arachidonate is converted into LTA₄ by the enzyme 5-lipoxygenase, when activated by ALOX5AP (arachidonate 5-lipoxygenase-activating protein). LTA₄ is metabolized further to LTB₄ by LTA₄ hydrolase or to LTC₄ by LTC₄ synthase. In response to inflammatory and immune stimuli, LTs are secreted into the extracellular space and subsequently bind to G-protein-coupled cell-surface receptors on their target cells [14].

Platelet-activating factor (PAF)

Platelet-activating factor has potent effects on other inflammatory cells such as macrophages, eosinophils and neutrophils. In view of this, increasing interest is being centred on the possible role of Platelet-activating factor in inflammation. This interaction may be of important pathophysiological importance in certain allergic disorders, especially in asthma and in other conditions in which eosinophils participate [15].

Disorder Associated to Inflammation

Asthma

The characteristic of asthma is persistent pulmonary inflammation with enhanced numbers of activated T-lymphocytes, eosinophils, monocytes, and resident tissue cells. These cells play an important role in establishment and maintenance of the chronic inflammatory process by releasing of cytokines, chemokines, and other proinflammatory mediators. There is enhancing evidence that reactive oxygen and nitrogen species are important mediators of the inflammatory process, providing a cellular mechanism that links oxidative stress and chronic inflammation [16].

Allergic inflammation

Characterization of the cellular and molecular events of allergic inflammation indicates a role for mast cells, T lymphocytes bearing the T helper type 2 (TH2) phenotype, and eosinophils. The prominence of eosinophils in the airways of asthmatics has drawn particular attention to their role in the pathophysiology of allergic inflammation [17].

Periodontal disease

In periodontal disease produces inflammation in the tooth-supporting tissues, requires a various approach when related to risk factors secondary to systemic disorders. The main causal agent of periodontal disease is bacterial plaque, which stimulate progressive tissue damage [18].

Granulomatous inflammation

Chronic granulomatous inflammation is a type four hypersensitivity reaction. A various type of agents, exogenous and endogenous may increases this type of chronic inflammation. These are mostly common in South Asian countries mostly due to high prevalence of tuberculosis and leishmaniasis. Tuberculosis used to be the leading cause of chronic granulomatous inflammation of the skin and some other part of body.

Mucositis & Cutaneous inflammation

Mucositis is a common adverse effect of cancer chemotherapy, which decreases the quality of life of patients during treatment. Mucositis is a weakening condition that can occur in all the area of the gastrointestinal tract; however, it most commonly affects the mucosa of the oral mucositis and intestinal mucositis. As the primary interface between the body and the external environment, the skin provides the first line of defence against traumatic injury and invasion by microbial pathogens [19].

Lung inflammation

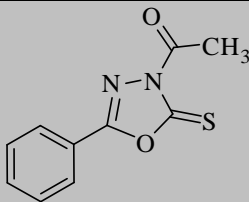
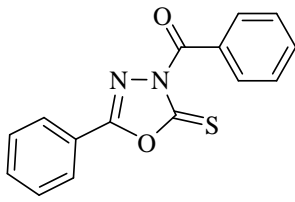
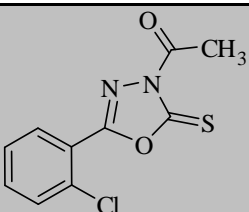
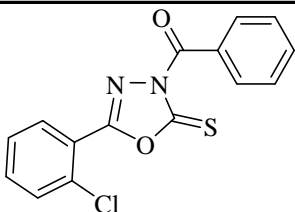
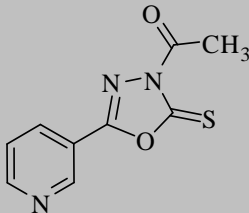
In neonatal inflammatory lung diseases with respiratory failure such as acute respiratory distress syndrome, an enhance expression of selectins, cytokines, chemokines and integrins, can be found to cause the invasion of inflammatory cells into lung tissue, which contribute to the degradation of surfactant films to hyaline membranes within the alveoli, and to the formation of a proteinaceous edema within the pulmonary interstitial space [20].

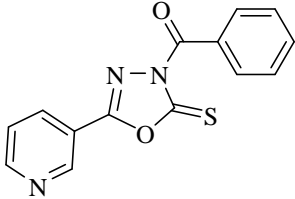
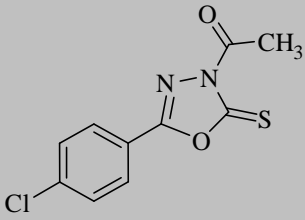
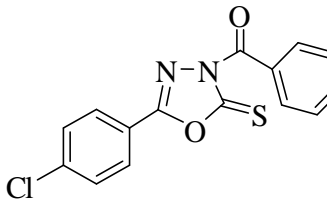
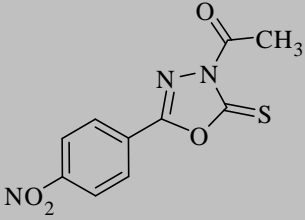
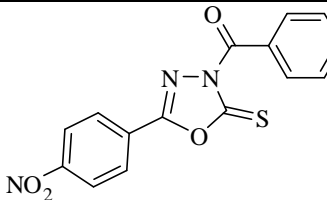
Arthrities

Arthritis is an inflammation of the joints of bone. All age's group of people including children and young adults can develop arthritis. The symptoms are intermittent swelling, pain, stiffness and redness in the joints of bone. Various types of arthritis, some of which are osteoarthritis, rheumatoid arthritis, spondylitis and infectious arthritis [21].

Synthesized Compounds

Table 1: Name, structure and molecular formula of title compounds

Sr. No.	Compound code	Name	Structure	Molecular formula
1.	IdA	1-(5-phenyl-2-thioxo 1,3,4-oxadiazol-3(2H)-yl) ethanone		C ₁₀ H ₈ N ₂ O ₂ S
2.	IdB	Phenyl(5-phenyl-2-thioxo-1,3,4-oxadiazol-3(2H)-yl) methanone		C ₁₅ H ₁₀ N ₂ O ₂ S
3.	IIdA	1-(5-(2-chlorophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)ethanone		C ₁₀ H ₇ ClN ₂ O ₂ S
4.	IIdB	(5-(2-chlorophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl) (phenyl)methanone		C ₁₅ H ₉ ClN ₂ O ₂ S
5.	IIIdA	1-[5-(pyridi-3-yl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl]ethanone		C ₉ H ₇ N ₃ O ₂ S

6.	III dB	Phenyl-[5-(pyridin-3-yl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl]methanone		C14H9N3O2S
7.	IV dA	1-[5-(4-chlorophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl]ethanone		C10H7ClN2O2S
8.	IV dB	[5-(4-chlorophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl](phenyl)methanone		C15H9ClN2O2S
9.	V dA	1-[5-(4-nitrophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl] ethanone		C10H7N3O4S
10.	V dB	[5-(4-nitrophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl] (phenyl)methanone		C15H9N3O4S

Pharmacological Screening

Male albino Wistar rats are used as Test animal which were procured from Central Drug Research Institute (C.D.R.I.), Lucknow and were kept under standard conditions like Temperature 22°C (\pm 3°C), Humidity 30-70 % . they having a standard pellet diet in animal house of Babu Banarasi Das National Institute of Technology & Management, Lucknow, UP, India. These Male albino Wistar rats were handle as per the protocol approved by the Institutional Animal Ethics Committee (IAEC) duly approved by CPCSEA (BBDNITM/IAEC/27/2011).

MATERIAL AND METHODS

- Animals: Male Albino Wistar rats of body weight 150-250 gm were used for the study. Animals

were maintained in clean polypropylene cages under standard conditions [Temperature 22°C (\pm 3°C), Humidity 30-70%]. Food (Pellets) and water given were purchased from local market of Lucknow.

- Drug and Chemicals: Standard drug and chemicals were purchased from local market of Lucknow.

All the animal studies were conducted in the accordance with the guidelines for the animal care. The study was cleared by ethical committee of the institution and certificate obtained.

Determination of Acute Toxicity

Acute toxicity study was performed by following Organization for Economic Co-operation and Development (OECD) guidelines. The test animals at 50 mg/kg dose levels showed no significant changes in behavior after the administration of an oral dose of test drugs. There was no significant change in body weight, food and water consumption, thus indicating the non toxic nature of synthesized compounds at 50 mg/kg body weight. The animals were monitored for next 14 days and no behavioral change observed. The result indicated that all of the tested compounds were non toxic and well tolerated by the experimental animals.

Anti-Inflammatory Activity

We can study anti inflammatory activity by Carrageenan induced Rat Paw oedema animal model. Carrageenans are a heterogeneous mixture of high-molecular-weight sulphated polysaccharides. carrageenan is obtained mainly from *Chondruscrispus*. When carrageenan is given in the paw of the rat by injection, Oedema develops.

Screening of Anti-Inflammatory Activity

Anti-Inflammatory activity by Carrageenan induced rat paw oedema method

Newly synthesized compounds were evaluated for their anti-inflammatory activity .The model used is carrageenan-induced acute paw oedemain rats. They all are given standard pellet diet and water was given *ad libitum*. Rats were placed in polypropylene cages under standard conditions. Rats were divided into different groups like control, standard and test groups, each group having five rats [22].

Group I: Diseased control (1% w/v carrageenan in 0.9% saline solution) intra peritoneal.

Group II: Diseased animals treated with reference drug Diclofenecsodium (10 mg/kg).

Group III: Diseased animals treated with test compounds in predetermined dose (10 mg/kg)suspended in CMC. The various groups were treated as follows:

Group 1: Control (normal saline, p.o.)

Group 2: Diclofenac sodium (10 mg/kg, p.o.)

Group 3: Compound IdA (10mg/kg, p.o.)

Group 4: Compound IdB (10mg/kg, p.o.)

Group 5: Compound IIdA (10mg/kg, p.o.)

Group 6: Compound IIdB (10mg/kg, p.o.)

Group 7: Compound IIIdA (10mg/kg, p.o.)

Group 8: Compound III dB (10mg/kg, p.o.)

Group 9: Compound IV dA (10mg/kg, p.o.)

Group 10: Compound IV dB (10mg/kg, p.o.)

Group 11: Compound V dA (10mg/kg, p.o.)

Group 12: Compound V dB (10mg/kg, p.o.)

These compounds were administered 30 min before carrageenan injection. Inflammation was induced in each group by injecting 0.1 ml of 1% carrageenan. A mark was put on the leg. The initial reading was taken at zero hour, that was immediately after injecting carrageenan, and the following readings were taken at 1, 2, 3, and 4 hours after carrageenan insertion. For comparison purpose, the volume of edema at various prefixed time intervals was measured plethysmography by mercury displacement method. The difference between zero reading and one of the subsequent readings provides the actual edema volume at that time. The mean paw volume at different times was calculated for all groups. Percentage reduction in oedema volume was calculated by using the formula.

$$\text{Percentage reduction} = \frac{V_0 - V_t}{V_0} \times 100$$

Where, V_0 = Volume of the paw of control at time 't'.

V_t = Volume of the paw of drug treated at time 't'.

From the data obtained, the mean oedema volume and percentage reduction in oedema was calculated [23].

Statistical Data Analysis

Table 2: Anti-inflammatory activity of synthesized 5-aryl-1,3,4- Oxadiazole 2(3H)thione Derivatives

Compounds	Mean paw volume \pm SEM(ml) and % Inhibition				
	Time after Carrageenan injection				
	0 hr	1 hr	2 hr	3 hr	4 hr
Control	0.150 \pm 0.006	0.262 \pm 0.013	0.326 \pm 0.012	0.372 \pm 0.012	0.406 \pm 0.023
IdA	0.148 \pm 0.008	0.192 \pm 0.015** (26.17%)	0.160 \pm 0.007** (50.92%)	0.136 \pm 0.010** (63.44%)	0.110 \pm 0.008** (72.90%)
IdB	0.146 \pm 0.006	0.228 \pm 0.009 (12.97%)	0.238 \pm 0.013** (26.99%)	0.232 \pm 0.021** (37.36%)	0.230 \pm 0.017** (43.35%)
II dA	0.138 \pm 0.008	0.182 \pm 0.010** (30.53%)	0.174 \pm 0.001** (46.62%)	0.156 \pm 0.010** (58.06%)	0.128 \pm 0.014** (68.47%)
II dB	0.144 \pm 0.006	0.214 \pm 0.016 (18.32%)	0.228 \pm 0.006** (30.06%)	0.224 \pm 0.013** (39.78%)	0.206 \pm 0.006** (49.24%)
III dA	0.134 \pm 0.006	0.214 \pm 0.012	0.192 \pm 0.013**	0.176 \pm 0.010**	0.162 \pm 0.013**

	006	(18.32%)	(41.10%)	(52.69%)	(60.10%)
III dB	0.142±0.010	0.202±0.006 (22.90%)	0.204±0.016** (37.42%)	0.184±0.009** (50.54%)	0.170±0.011** (58.13%)
IV dA	0.140±0.007	0.190±0.008** (27.48%)	0.164±0.008** (49.69%)	0.152±0.006** (59.14%)	0.122±0.008** (69.95%)
IV dB	0.138±0.07	0.228±0.015 (12.98%)	0.226±0.012** (30.67%)	0.210±0.017** (43.54%)	0.202±0.010** (50.24%)
V dA	0.138±0.011	0.216±0.018 (17.56%)	0.176±0.006** (46.01%)	0.162±0.011** (56.45%)	0.132±0.013** (67.48%)
V dB	0.148±0.009	0.214±0.014 (17.56%)	0.236±0.013** (27.61%)	0.224±0.007** (39.78%)	0.220±0.008** (45.51%)
Standard	0.148±0.017	0.148±0.017** (43.51%)	0.130±0.014** (60.12%)	0.092±0.006** (75.26%)	0.076±0.007** (81.13%)

Values are expressed as mean ± SEM; n=5

P<0.01 compared with vehicle treated group using one way ANOVA followed by Dunnett's test.

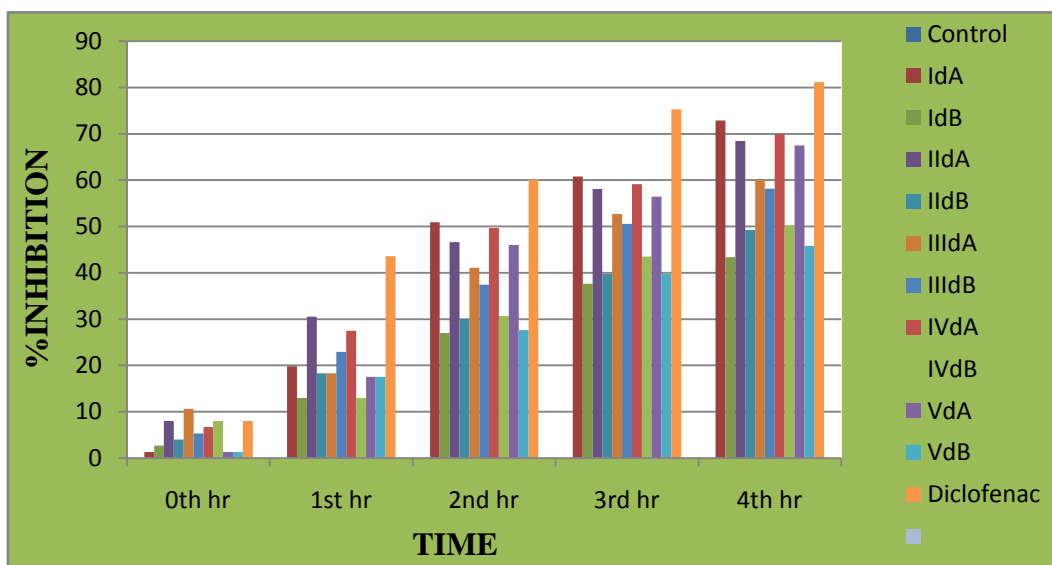


Figure 2: Anti-inflammatory activities of synthesized compounds

5-aryl-1,3,4-oxadiazole-2(3H) thione of substituted methoxy and benzy were evaluated for their influence on the anti-inflammatory.

RESULT AND DISCUSSION

Anti-Inflammatory Activity

Inflammation is associated with pathophysiology of various clinical conditions like arthritis, cancer and vascular diseases. A number of synthetic compounds are used in various modern systems of medicine to treat symptoms of pain and inflammation. The carrageenan induced hind paw edema is a standard experimental model. Carrageenan induced paw edema is characterized by biphasic events, with involvement of different inflammatory mediators. An insight into the anti-inflammatory activity with respect to the chemical structure reveals that compounds IdA, IIdA, IIIdA, IVdA and VdA bearing methoxy group exhibited good activity (72.90%**, 68.47%**, 60.10%**, 69.95%** and 67.48%**) at a dose of 10mg/kg in comparison to standard drug Diclofenac sodium (81.13%**). The compounds showed significant ($P < 0.01$) inhibition of the edema from all the phases of inflammation, by probably inhibiting the different aspects and chemical mediators of inflammation.

The administration of all other derivatives of 5-aryl-1,3,4-oxadiazole-2(3H) thione (10 mg/kg) showed moderate inhibition of the edema from all the phases of inflammation. The development of oedema is biphasic. The initial phase because of the release of histamine, serotonin, 5-hydroxy tryptamine and kinins in the first hour and second phase is because of the release of prostaglandin like substances in 2-3 hr.

CONCLUSION

On the basis of literature survey, various derivatives of 5-aryl 1,3,4-oxadiazole-2(3H)thione derivatives were evaluated for anti-inflammatory activity. The Anti-inflammatory activity was carried out in carrageenan induced rats paw edema model and compounds (IdA) $0.110 \pm 0.008^{**}$, (IIdA) $0.128 \pm 0.014^{**}$, (IIIdA) $0.162 \pm 0.013^{**}$, (IVdA) $0.122 \pm 0.008^{**}$ and (VdA) $0.132 \pm 0.013^{**}$ showed appreciable anti-inflammatory activity in comparison to standard drug Diclofenac $0.076 \pm 0.007^{**}$. The benzoxy substituted oxadiazole compounds (IdB) $0.230 \pm 0.017^{**}$, (IIdB) $0.206 \pm 0.006^{**}$, (IIIdB) $0.170 \pm 0.011^{**}$, (IVdB) $0.202 \pm 0.010^{**}$ and (VdB) $0.220 \pm 0.008^{**}$ displayed less activity as compared to other compounds.

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