Synthesis, Characterization of Some New [1, 3, 4]-Oxadiazole Derivatives as Anti-Inflammatory Agents

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ABSTRACT :
The present study focuses on the design, synthesis, and characterization of novel [1, 3, 4]-oxadiazole derivatives with potential biological applications. Acid hydrazides derived from 2-(4-isobutylphenyl)propanoic acid and 4-methylthiophenylacetic acid were subjected to cyclization with carbon disulfide under basic conditions to afford [1,3,4]-oxadiazol-2-thiones. These intermediates were further reacted with formaldehyde and various primary aromatic and secondary amines via Mannich-type aminomethylation, resulting in a series of new Mannich bases. The purity of the synthesized compounds was verified by thin-layer chromatography (TLC). Structural elucidation was carried out using spectroscopic techniques including IR, ^{1}H NMR, ^{13}C NMR, 2D NMR, and mass spectrometry. The synthesized compounds were evaluated for their antimicrobial activity, and selected derivatives were also tested for anti-inflammatory potential. The results demonstrated promising biological activities, indicating the potential of these oxadiazole-based compounds for further pharmacological development.
Keywords: [1,3, 4]-OXADIAZOLE, Mannich bases, TLC, IR, ^{1}H NMR, ^{13}C NMR, 2D NMR, Mass spectral data, anti-inflammatory activities.

I. INTRODUCTION

Heterocycles in drug discovery

Nowadays, the entire pharmaceutical industry is faced with the challenge of increasing productivity and innovation. The major hurdles are the increasing costs of research and development and a simultaneous stagnating number of new chemical entities (NCEs). The cause of this innovation deficit is definitively not the biology. Decoding of the human genome has led to a wealth of drug targets. With more than 30,000 human genes, the assumption is that at least 1,000 are significantly involved in the emergence and course of disease. Furthermore, because each of these genes is linked to the function of between five and ten proteins, the conclusion is that there might be 5,000–10,000 targets for new drugs[1]. Despite the successful introduction of protein therapeutics and the promise of gene therapy, major pharmaceutical companies are still focused on the discovery and development of low-molecular weight compounds. Hence, the challenge is to select the most drugable targets and to find the corresponding drug-like molecules, substances that not only interact with the target, but also have specific pharmacokinetic and toxicological properties, that allow them to be developed as a drug. Medicinal chemistry as a scientific discipline has introduced several new techniques over the last few years in order to speed up the drug discovery process, such as combinatorial chemistry, microwave-assisted organic synthesis (MAOS) and high-throughput purification [2]. Despite this steady increase in R & D, the number of NCEs reaching the market has actually decreaseddramatically.It seems clear that selecting appropriate molecules to synthesize is one of the most troublesome questions. It has been estimated that the number of possible molecules with a molecular weight of less than 500 Da is 10^{200}, of which only 10^{60} may possess drug-like properties. The proportion of these drug-like molecules synthesized to date has been estimated as one part in 10^{57}, or roughly the ratio of the mass of one proton to the mass of the sun! The issue is therefore the selection of new molecules from this vast universe, which have the potential to be biologically active [3].

It is well documented that vast majority of Pyrazole[4-12], Quinazoline[13]and Pyrazoline[14] derivatives are known to possess pharmacologically proven therapeutic potentials. Novel and Efficient method of synthesis for Nitrogen containing heterocyclic molecule still represent highly pursued target for antimicrobial, antimarerial and other biological activities. A wide investigation or evolution, development of active analog and random discovery is a valuable and employed method for drug discovery.

II. EXPERIMENTAL WORK

Experimental Procedure:

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 (CDCb/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).

Present Work

Although the demand for new chemical materials and biologically active molecules continues to grow, chemists have hardly begun to discover the enormous pool of potentially active compounds. In the scenario of a persistent request especially from the pharmaceuticals companies for better drugs, it has become a challenging task for medicinal chemists to prepare new patentable molecules that combine high activity and selectivity, drug-likeness, and good pharmacokinetic properties.

As part of our continuing interest in the synthesis of biologically active compounds we have successfully synthesized such derivatives which consist of distinct pharmacophores; [1,3,4]oxadiazole , each certainly, possessing a wide range of biological and pharmacological activities. [1,3,4] oxadiazole scaffold derivatives are an important class of potential organic molecules in medicinal chemistry due to their extensive range of activity such as neuron protective, anti-convulsive, anti-glutamate, anti-malarial, anthelmintic, anti-tubercular, analgesic, anti-microbial, and anti-cancer to name a few . In this context, synthetically accessible molecules having new [1,3,4] oxadiazole scaffold with promising biological profile have attracted the attention of medicinal organic chemists for their applications in potential chemotherapeutics.

Current working Scheme

The acid hydrazides **(1)** were prepared by esterification of 4-methylthiophenyl acetic acid and 2-(4-isobutylphenyl) propanoic acid with absolute ethanol in the presence of catalytic amount of cone, sulfuric acid followed by treatment with hydrazine hydrate in absolute alcohol. The acid hydrazides were then subjected to cyclization with carbon disulphide in the presence of potassium hydroxide in absolute alcohol to afford the corresponding 5-substituted-[l,3,4]-oxadiazole-2[3/f]-thiones**(2).** The resultant oxadiazoles **(2)** were further converted into their corresponding Mannich bases **(3,4**and **5)** on aminomethylation with formaldehyde and various secondary amines/primary aromatic amines.

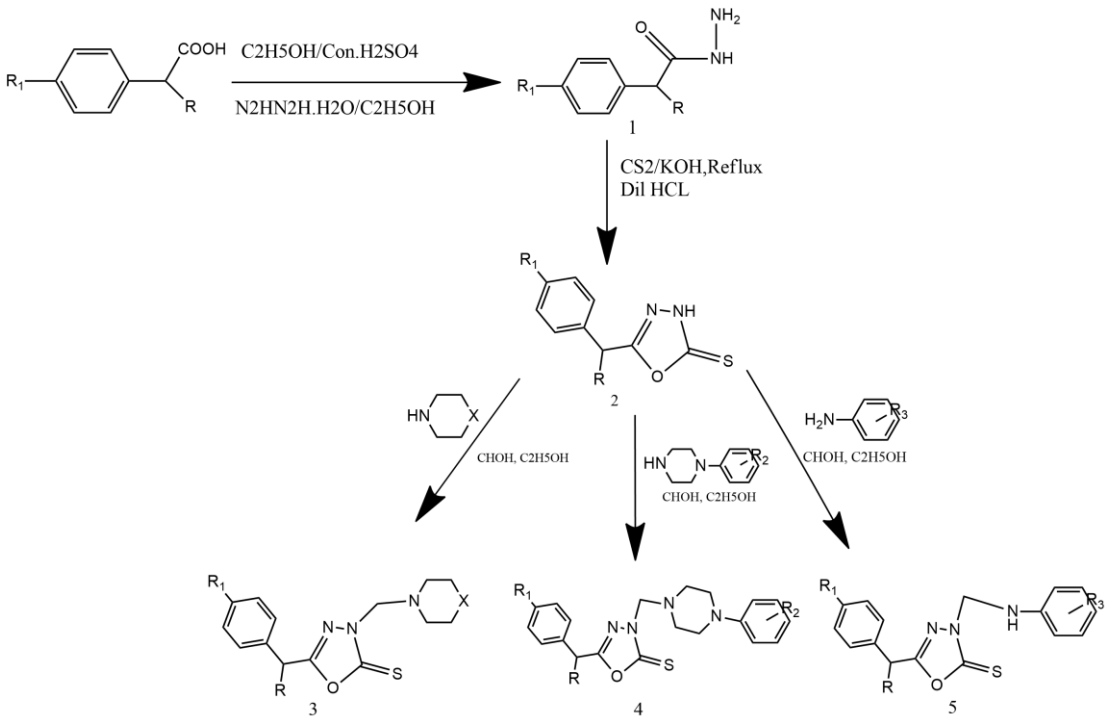


Figure – 1: Synthesis Scheme for 5-substituted-[l,3,4]-oxadiazole-2(3//)-thiones

Compd	R	R1	R2	R3	X	Mol. Formula
3a	H	SCH_3	----	----	O	$C_{15}H_{19}N_3O_2S_2$
3b	H	SCH_3	----	----	NH	$C_{15}H_{20}N_4OS_2$
4a	H	SCH_3	4-NO_2	---	---	$C_{21}H_{23}N_5O_3S_2$
4b	H	SCH_3	4-OCH_3	---	---	$C_{22}H_{26}N_4O_2S_2$
5a	H	SCH_3	----	2,4-Cl	---	$C_{18}H_{17}N_3OSCl_2$
5b	H	SCH_3	----	4-NO_2	---	$C_{18}H_{18}N_4O_3S$

General Procedure for the Preparation of Arylhydrazines (1)

The ethyl esters were synthesized by refluxing substituted aromatic acids with an excess of 100% ethanol, accompanied by a few drops of concentrated sulphuric acid, following the standard esterification procedure. The resultant esters were deemed pure by thin-layer chromatography (TLC).
The combination of ethyl ester of substituted aromatic acids (0.1 mol) and hydrazine hydrate (0.2 mol) was subjected to reflux in absolute alcohol (50 mL) for 8 hours. The surplus solvent was further distilled at decreased pressure, and the concentrated solution was then quenched in ice-cold water. The solid was filtered, washed, and dried. The crude product underwent purification by recrystallisation from ethanol.

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

A combination of aroyl hydrazide (1) (0.1 mol), KOH (5.6 g, 0.1 mol) in absolute alcohol (50 mL), and CS2 (15.2 g, 0.2 mol) was placed in a round-bottom flask and refluxed for about 4 hours until the release of hydrogen sulphide halted. The reaction mixture was allowed to cool to ambient temperature and then diluted with water. The product obtained upon acidification with weak hydrochloric acid was filtered, meticulously rinsed with cold water, and recrystallised from ethanol.

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

A solution of 5-aryl-2-mercapto-[1,3,4]-oxadiazole-2-thione (10 mmol) in ethanol (15 mL) was subjected to the addition of a combination of formaldehyde (0.45 g, 15 mmol) and a secondary amine (10 mmol) in 10 mL of ethanol, while stirring. Following the whole addition, stirring was maintained overnight at ambient temperature. The precipitated solids were subjected to filtration, rinsed with water, and then dried. The crude product underwent recrystallisation from ethanol.

BIOLOGICAL ACTIVITY

ANTI-INFLAMMATORY ACTIVITY

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 [15].
In the present study, the anti-inflammatory activity of six newly synthesized compounds was determined by carrageenan induced paw edema method [16-17].

Animals

Wistar albino rats of either sex weighing 180-250 g were used for the experiment. They were housed in the clean polypropylene cages and kept under room temperature, relative humidity (60-70 *%)* in 12 h of light-dark cycle. The animals were given standard laboratory diet and water *ad libitum.* Food was withdrawn 12 h before and during experimental hours.

III. RESULTS AND DISCUSSION

Formations of the Mannich bases (1 and 2) were also confirmed by the elemental analyses, IR, ^{1}H NMR, ,^{13}C NMR, 2D NMR and Mass spectral data. The IR spectrum of Mannich base 3a showed the absence of absorption bands corresponding to the NH group of the parent oxadiazole. It showed absorption bands at 3045 for aromatic C-H, 1617 cm-1 for C=N, 1330 cm-1 for C=S and 1249 cm-1 for C-O stretching vibrations. The 400 MHz ^{1}H NMR spectrum 3a showed the signals corresponding to the NH/SH tautomeric proton was absent and a new singlet for N-CH2-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 SCH3 and CH2 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 3a 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 3a 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 $C_{15}H_{19}N_3O_2S_2$. 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 **(3a, 4a** and **5a)** 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 elemental analyses, IR, ^{1}H NMR, ,^{13}C NMR, and Mass spectral data. The IR spectrum of Mannich base **3a (Fig. 3.1)** showed the absence of absorption and corresponding to the NH group of the parent oxadiazole. It showed absorption bands at 3045 for aromatic C-H, 1617 cm-1 for C=N, 1330 cm-1 for C=S and 1249 cm-1 for C-O stretching vibrations.

The 400 MHz ^{1}H NMR spectrum **3a (Fig. 3.2)** showed the signals corresponding to the NH/SH tautomeric proton was absent and a new singlet for N-CH2-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 SCH3 and CH2 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 **3a (Fig. 3.4)** 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 mass spectrum **(Fig. 3.5)** of **3a** 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 $C_{15}H_{19}N_3O_2S_2$. The base peak was observed at m/z 100 due to the formation of morpholinomethyl cation.

The IR spectrum of the Mannich base **4a (Fig. 3.6)** showed characteristicabsorption bands at 3045 cm^{-1}for aromatic C-H, 2742-2891 cm^{-1}for aliphatic/alicyclic C-H, 1579 cm^{-1}for C=N and 1342 cm^{-1}for C=S groups. The asymmetric and symmetric stretching vibrations of NO**2** group are observed at 1435 and 1112 cm^{-1}respectively.

The 400 MHz 'H NMR spectrum of **4a** is depicted in **Fig. 3.7.** The spectrum 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- 4-nitrophenylpiperazine ring appeared as two doublets centred at *δ*6.99 and 8.02 with *J*= 9.6 Hz. The mass spectrum **(Fig.3.8)** of **4a** 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** is shown in **Fig. 3.9.** The spectrum showed a broad absorption band at 3043 cm^{-1} for its N-H moiety. The other characteristic absorption bands at 1579 cm^{-1} for C=N, 1330 cm $^{-1}$ for C=S and 696 cm $^{-1}$, 746 cm $^{-1}$ for C-Cl groups, were also observed in the spectrum. The 400 MHz !H NMR spectrum of Mannieh base **5 (Fig. 3.10)** showed a 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 **5a(Fig. 3.11)** 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 **(Fig.3.12)** 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 $C_{17}H_{25}C_{I2}N_3OS_2$.

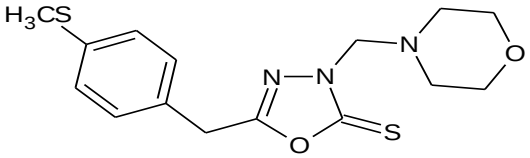
Table 1: Physical Characterization data of 5-substituted-[l,3,4]-oxadiazole Mannich bases 3,4 and 5.

Compd	R	R1	R2	R3	X	Mol. Formula	Mol. Wt.	M.P ºC	% yield
3a	H	SCH_3	----	----	O	$C_{15}H_{19}N_3O_2S_2$	337	88–90	62
3b	H	SCH_3	----	----	NH	$C_{15}H_{20}N_4OS_2$	336	128-130	67
4a	H	SCH_3	4-NO_2	---	---	$C_{21}H_{23}N_5O_3S_2$	457	168-170	82
4b	H	SCH_3	4-OCH_3	---	---	$C_{22}H_{26}N_4O_2S_2$	442	135-137	67
5a	H	SCH_3	----	2,4-Cl	---	$C_{18}H_{17}N_3OSCl_2$	393	140-142	85
5b	H	SCH_3	----	4-NO_2	---	$C_{18}H_{18}N_4O_3S$	370	131-133	68

SPECTRAL CHARACTERIZATION

5-(4-Methylthiobenzyl)-3-[(4-methylpiperazin-l-ylmethyl)-[l,3,4]-oxadiaz0le-2(3H)' Thione3

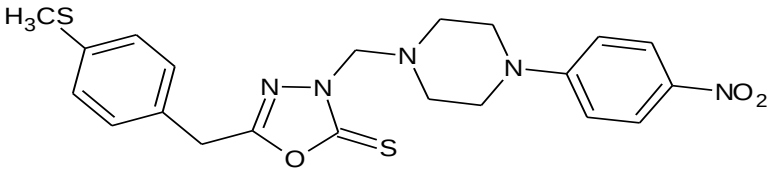
Structure:



Molecular formula: $C_{15}H_{19}N_3O_2S_2$

Molecular weight:336

5-(4-methylthiobenzyl)-3-{[4-(4-nitrophenyl)piperazin-l-yljmethyl}-[13,4J-oxadiazoie-2(3//)-thione (4)

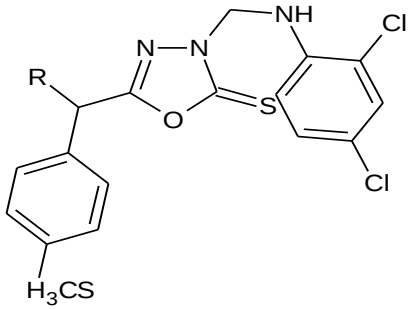


Molecular formula: $C_{21}H_{23}N_5O_3S_2$

Molecular Weight: 457

5-(4-methylthiobenzyl)-3-{[4-(2,4-dichlorophenyl)]methyl}-[l,3,4]-oxadiazole-2(3/i)-thione (5)

Structure:



Molecular formula: $C_{17}H_{15}N_3OS_2Cl_2$

Molecular weight:411

The tested compounds showed anti-inflammatory activity ranging from 50.17% to 72.71%, whereas standard drug diclofenac sodium showed 73.66 % inhibition after 3h (Table 2). The highest activity (72.71%) was found for the Mannich base, **5a.**

The anti-inflammatory activity of oxadiazole Mannich bases derived from 4-thiomethylphenyl acetic acid was in the range of 50.17% to 72.71% inhibition. The highest activity (72.71%) was found for the Mannich base, 5a.

The results were expressed as % inhibition of oedema over the untreated controlgroup. The results of anti-inflammatory studies are given in Table 2.

The tested compounds showed anti-inflammatory activity ranging from 30.68% to 72.71 %, whereas standard

drug diclofenac sodium showed 79.66 % inhibition after 3h (Table 2).

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
3a	15	0.282 ± 0.0025	50.17
3b	15	0.238 ± 0.0022	57.37
4a	15	0.232 ±0.0021	59.61
4b	15	0.406 ±0.0031	30.68
5a	15	0.106 ± 0.0034	72.71
5b	15	0.310 ±0.0019	44.63
Control	0.1 ml/kg	0.156 ±0.031	----------
Standard	15	0.015 ± 0.0017	79.66

Result was Mean ± SD, n = 6

CONCLUSION

Six derivatives of [l,3,4]-oxadiazole Mannich bases, synthesised from ibuprofen and 4-methylthiophenyl acetic acid, were developed with the aim of enhancing anti-inflammatory drugs. Compounds 5a exhibited anti-inflammatory action, followed by 4a, 3b, 5b, 3a, and 4b, when compared to the standard medication diclofenac at a dosage of 15 mg/kg p.o. in the carrageenan-induced paw oedema test in rats.

Numerous [l,3,4]-oxadiazole Mannich bases synthesised from ibuprofen and 4-methylthiophenyl acetic acid were created with the aim of enhancing anti-inflammatory medicines. It is noteworthy that compounds 3a, 4a, and 5a exhibited anti-inflammatory action when compared to the conventional medicine, diclofenac, administered at 15 mg/kg orally, with 5a demonstrating the highest efficacy.

REFERENCES

1. Drews, J. Science 2000, 287, 1960; (b) Wess, G.; Urmann M.; Sickenberger, B. Angew. Chem. Int. Ed. 2001, 40, 3341.
2. Lombardino, J. G.; Lowe III, J. A. Nat. Rev. Drug Discov. 2004, 3, 853.
3. Kolbn H. C.; Finn, M. G.; Sharpless, K. B. Angew. Chem. Int. Ed. 2001, 40, 2004.
4. Vijay V. Dabholkar and Faisal Y. Ansari, J. Serb. Chem. Soc., 2009, 74, 1219–1228.
5. G. Saravanan, V. Alagarsamy, G. Chanukya, Int. J. of Pharma and Bio Sci., 2010,1,1-8.
6. K. Narasimha Sarma, M.C.S.Subha and K.Chowdoji Rao, E-J. of Chem, 2010,7, 745-750.
7. Vertika Gautam, Viney Chawla, Pankaj K. Sonar and Shailendra K. Saraf, E-J of Chem, 2010 7, 1190-1195.
8. Rafat M. Mohareb, Hanaa Y. Hana, Acta Pharm., 2008, 58, 29–42.
9. R.N. Sharma, K.P.Sharma, S.N.Dixit, Int.J. ChemTech Res. 2010, 2, 800-806.
10. Vetrivel Nadaraj and SenniappanThamaraiSelvi, Der Pharma Chemica, 2010, 2, 315-321.
11. Raj Narayan Sharma1, K.P.Sharma2 S.N. Dikshit3, Der Pharma Chemica, 2010, 2, 38-45.
12. Nirav K Shah, Manish P Patel and Ranjan G Patel, India J Chem, 2009, 48B, 1170-1173.
13. Patel NB, Patel JC. Synthesis and antimicrobial activity of Schiff Bases and 2-azetidinones derived from quinazolin-4(3H)-one.Arabian Journal of Chemistry. 2011; 4: 403-411.
14. Y. Hiroyuti, O. Mocoto, et al.; Eur. Pat. Appl. Ep 295695 (Cl. C07D 401/6) (1988); Chem. Abstr., 1989,111, 23510.
15. Ferrero-Miliani, L. et al. (2007). *Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation.* Clinical and Experimental Immunology, 147(2), 227–235.
16. *Winter, C.A., Risley, E.A., & Nuss, G.W. (1962).* Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs.***Proceedings of the Society for Experimental Biology and Medicine****, 111(3), 544–547.*
17. *Vinegar, R., Schreiber, W., & Hugo, R. (1969).* Biphasic development of carrageenin edema in rats.***Journal of Pharmacology and Experimental Therapeutics****, 166(1), 96–103.*