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Synthesis and Evaluation of Antimicrobial and Anti-inflammatory Activity of Oxadiazole Derivatives

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ABSTRACT:-

The synthesis, structure and biological activity of Oxadiazole derivatives have long been the focus of research interests in the field of Medicinal Chemistry. A number of Oxadiazole derivatives have been reported to possess interesting biological activities such as Antimicrobial, Anti-inflammatory and Antifungal activities etc.

In the present proposal, aromatic aldehyde was made to react with ethyl acetoacetate in presence of ammonia to yield 1, 4-dihydropyridine derivatives. 1, 4-dihydropyridine was refluxed in presence of hydrazine and ammonia to yield offer corresponding hydrazides. Hydrazide was refluxed with an aromatic acid along with addition of phosphorous to offer final compounds.

All synthesized compound were characterized by IR, H^1-NMR and elemental Analysis.

All the compounds were evaluated for Antibacterial and Anti-inflammatory at the concentration of 200 µcg/mL by using cup-plate agar diffusion method. The activity was carried out on different micro-organisms (E. coli, S. aureus, A. niger, C. albicans) measured in terms of zone of inhibition and compared the standard drug Ciprofloxacin and Amphotericin B for antimicrobial activity.

All the newly synthesized derivatives were screened for Anti-inflammatory activity by an in-vitro method of Inhibition of protein denaturation using Zaltoprofen as a standard.

These compounds with the suitable molecular modification may prove as a drug of choice in the treatment of microbial infectious disease in future.

KEYWORDS: Anti-inflammatory, Antimicrobial and Oxadiazole.

INTRODUCTION

1.0 INTRODUCTION:

The discipline of medicinal chemist is devoted to the discovery and development of new agents for treating diseases. Most of this activity is directed to natural or synthetic organic compounds. Inorganic compounds continue to be important in therapy, e. g. trace elements in nutrition therapy, antacids and radiopharmaceuticals, but organic molecules with increasingly specific pharmacological activities are clearly dominant. Development of organic compounds has grown beyond traditional synthetic methods. The process of establishing new pharmaceuticals is exceeding complex and involves the talents of the people from a variety of disciplines including chemistry, biochemistry, molecular biology, physiology, pharmacology, pharmaceutics and medicine. Medicinal chemistry itself is concerned mainly with organic, analytical and biochemical aspects of this process, but the chemist must interact productively with those in other disciplines. Thus, medicinal chemist occupies a strategic position at the interface of chemistry and biology. Now a day's medicinal chemists are at the forefront of innovation, blending synthetic chemistry, molecular modeling, computational biology, structural genomics and pharmacology to discovery and design new drugs and investigate their interaction at the cellular level. Many efforts are being made in the design and development of novel drugs from synthetic origin. Thus, there is growing interest in the pharmacological potential.

Oxadiazoles, Triazoles derivatives attracted organic chemists very much due to their biological and chemotherapeutic importance. Triazolo-oxadiazoles and related fused heterocycles are of interest as potential bioactive molecules. In many cases, heterocyclic fusion of ring resulted in compounds with wide spectrum of biological activities. Some of the activities possessed by hetero fused Triazolo-oxadiazoles include antimicrobial, antidiabetic, antifungal, anticancer, anti-inflammatory, antitubercular activity etc.

1.1 ANTIBACTERIAL ANTIBIOTICS:

An antimicrobial agent is anything that can kill or inhibit the growth of bacteria, such as high heat or radiation or a chemical. Antibacterial chemicals can be grouped into three broad categories like antibacterial drugs, antiseptics, and disinfectants. Bacteria are single celled organisms that live in and around us. Bacteria may be helpful, but in certain conditions may cause illness like throat, most ear infections, and bacterial pneumonia. Antibacterial drugs are used in relatively low concentrations in or upon the bodies of organisms to prevent or treat specific bacterial diseases without harming the host organism. Unlike antibacterial drugs, antiseptics and disinfectants are usually nonspecific with respect to their targets, i.e. they can kill or inhibit a variety of microbes.

Basis of Antimicrobial Action:

Various antimicrobial agents act by interfering with (1) cell wall synthesis, (2) plasma membrane integrity, (3) nucleic acid synthesis, (4) ribosomal function, and (5) folate synthesis.[1]

Biochemical Basis of Antimicrobial Action:

Bacterial cells grow and divide, replicating repeatedly to reach the large numbers present during an infection or on the surfaces of the body. To grow and divide, organisms must synthesize or take up many types

of biomolecules. Antimicrobial agents interfere with specific processes that are essential for growth and/or division. They can be separated into groups such as inhibitors of bacterial and fungal cell walls, inhibitors of cytoplasmic membranes, inhibitors of nucleic acid synthesis, and inhibitors of ribosome function. Antimicrobial agents may be either bactericidal, killing the target bacterium or fungus, or bacteriostatic, inhibiting its growth. Bactericidal agents are more effective, but bacteriostatic agents can be extremely beneficial since they permit the normal defenses of the host to destroy the microorganisms.[2]

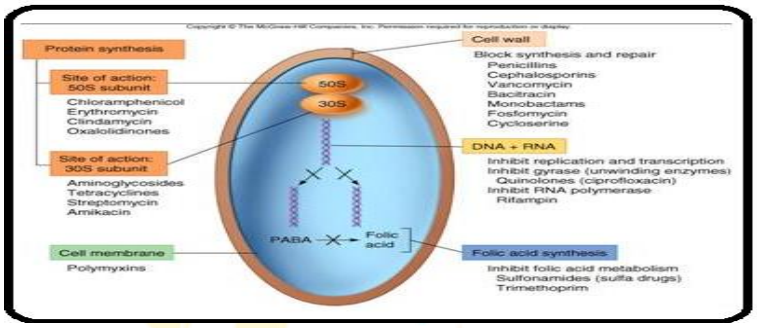


Fig. 1.1.1 Sites of action of different antimicrobial agents.

Inhibition of Bacterial Cell Wall Synthesis: Bacteria are classified as Gram-positive and Gram-negative organisms on the basis of staining characteristics. Cell walls of Gram-positive bacteria contain peptidoglycan and teichoic or teichuronic acid, and the bacterium may or may not be surrounded by a protein or polysaccharide envelope.Cell walls of Gram-negative bacteria contain peptidoglycan, lipopolysaccharide, lipoprotein, phospholipid, and protein. This layer is essential for the survival of bacteria in hypotonic environments; loss or damage of this layer destroys the rigidity of the bacterial cell wall, resulting in death.[3]

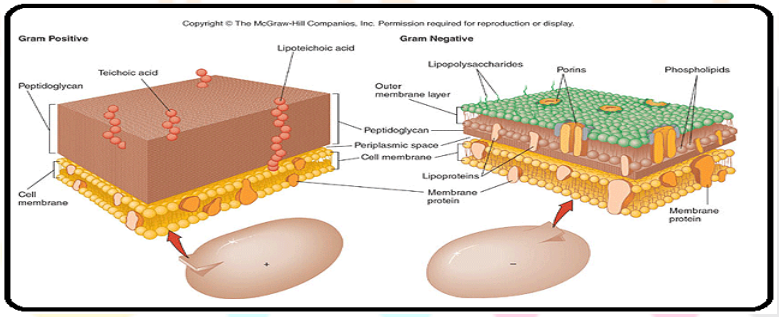


Fig. 1.1.2 Outer wall of Gram-positive and Gram-negative species and detail of poring channels of Gram-negative bacteria.

Classification

By structure and more importantly by mechanism of action, the effect of an antimicrobial on a susceptible bacterium is either to destroy it (bactericidal effect), or to prevent their multiplication or growth (bacteriostatic effect) or prevent their pathogenic action.[4]

Table No.1.1.1Signs and Symptoms[5]

Signs or symptoms	Examples
Systemic	Fever, elevated WBC count, increase in neutrophils (often referred to as left band shift)
At the local site	Redness (erythema), purulent drainage, presence of WBCs in normally sterile fluids (CSF or urine in males).
Organ specific (will help but do not confirm)	Flank pain (pyelonephritis), stiff neck (meningitis), lower right abdominal pain (appendicitis)
Predisposing factors	Surgery, immune status, etc.

Table No. 1.1.2: Three Basic Ways Antimicrobial Therapy

Type	When	Spectrum
Empiric	Organism not yet identified and have sent off cultures	Broad
Definitive	Know organism and susceptibilities	Narrow
Preventative (prophylactic)	To prevent initial or recurrent infection in susceptible host (E.g., Bactrim in HIV+ patient to prevent pneumocystis carinii pneumonia)	Depends on situation

1.2 INFLAMMATION:

Inflammation is defined as the local response to living mammalian tissues to injury due to any agent. Specifically, it is a series of molecular and cellular responses acquired during evolution designed to eliminate foreign agents and promote repair of damaged tissues.[6]

Types of Inflammation:

Depending upon the defense capacity of the host and duration of response.

Acute Inflammation:

It is a short duration and represents the early body reaction and is usually followed by repair. Its main features are accumulation of fluid and plasma at the affected site, intravascular activation of platelets, polymorphonuclear neutrophils as inflammatory cells.

Chronic Inflammation:

It is of longer duration and occurs either after the causative agents of acute inflammation persists for a long time, or the stimulus induces chronic inflammatory cells such as lymphocytes, plasma cells and macrophages.

This process has two major components:

- Vascular changes: Increased blood flow (vasodilatation) and structural changes that permit plasma proteins to leave the circulation (increased vascular permeability).
- Cellular events: Emigration of the leukocytes from the microcirculation and accumulation in the focus of injury.

Chemical Mediators of Inflammation:

These are also called as permeability factors or endogenous mediators of increased vascular permeability. The substances acting as chemical mediators of inflammation may be released from the cells, the plasma or damaged tissue itself.

Table No. 1.2.1 Some commonly used NSAIDs

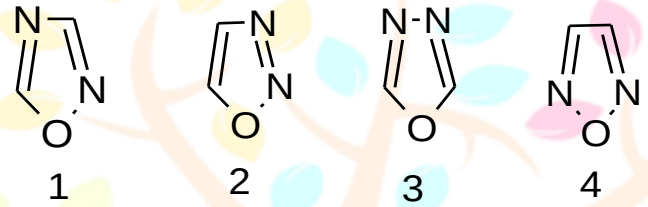
Drug	Plasma half-life (hours)	Comments
Non-selective COX inhibitors		
Aspirin	**3-5**	-
Diflunisal	**8-13**	Less GIT irritation than aspirin; long acting (is related to aspirin)
Ibuprofen	**2**	First-choice drug; lowest incidence of unwanted effects
Fenbufen	**10**	A pro-drug, activated in the liver; less risk of GIT, reactions, more risk of skin reactions
Naproxen	**14**	The same chemical class as ibuprofen but rather more potent; reasonable efficacy, moderate risk of adverse reactions
Mefenamic acid	**4**	Only moderate anti-inflammatory action; diarrhoea likely; hemolytic anaemia has been reported; possible interaction with warfarin; skin reactions can occur
Nabumetone	**12**	A pro-drug, activated in the liver; adverse effects less marked than with aspirin, Antipyretic action more marked
Diclofenac	**1-2**	Moderate potency; moderate risk of adverse GIT effects
Sulindac	**7**	A pro-drug interconvertible with active sulfide metabolite; moderate risk of side-effects.
Indomethacin	**2**	Potent inhibitor of COX in vitro; high incidence of non-GIT side-effects; headache, dizziness, etc.
Tolmetin	**5**	Efficacy as for ibuprofen; moderate risk of adverse effects
Piroxicam	**45**	GIT irritation in 20% of patients; tinnitus; rashes
Tenoxicam	**72**	Steady-state plasma concentration only after 2 weeks
COX-2 inhibitors		
Celecoxib	**11**	New compound; markedly less GIT toxicity

THEORETICAL DISCUSSION

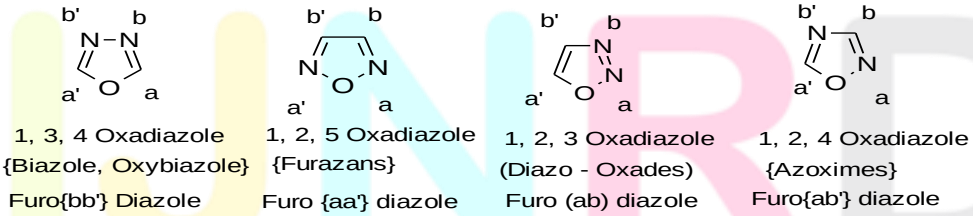
OXADIAZOLE:

Oxadiazole having a five-member heterocyclic ring which has two nitrogen atoms with an oxygen atom are considered to be an important class of compounds in medicinal chemistry because of their interesting diversified biological application. Literature survey revealed that a minor modification in the structure can result in qualitative as well as quantitative changes in the activity, convinced us to begin on the synthesis of various new 1, 3, 4- Oxadiazole derivatives with the aim of having improved activity and lesser toxicity. During the past few years, considerable evidence has been accumulated that demonstrates the efficacy of 1, 3, 4-oxadiazoles having anti-tubercular, and anti-hypoglycemic activity, anti-inflammatory activity, anticancer activity, antibacterial activity.[7]

Oxdiazole is consider to be derived from furan by replacement of two methene (CH=) group by two pyridine type nitrogen (-N=). There are four possible isomers of Oxadiazole (1, 2, 3, and 4) depending on the position of nitrogen atom in the ring and are numbered as shown.[29]



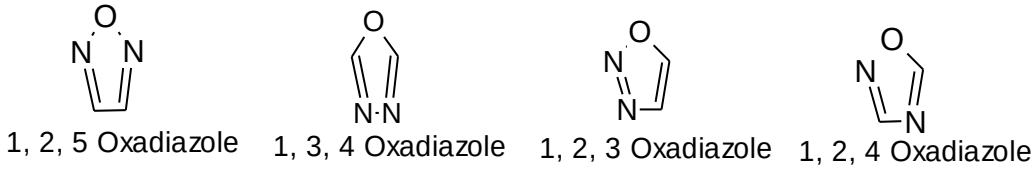
Compounds having a five-member ring containing one oxygen and two nitrogens are called oxadiazole or in the older literature furadiazole name for Oxadiazole ring such as ‘Azoxime’ (1, 2, 4 oxadiazole), ‘Furazan’ for (1, 2, 5 oxadiazole) has gain acceptance, as a affect the literature is complete of diversity of name for this molecule. Amongst these or “Oxybiazole”, “Diazoxole” “Furo (bb′) diazole and “Biozole”. The systematic name of 1, 3, 4-oxadiazole has gradually become prevalent and is used exclusively.[8]



CHEMISTRY [9-10]

Oxadiazole is a heterocyclic aromatic chemical compound with the molecular formula

$C_2H_2N_2O$. There are four isomers of oxadiazole: 1,2,4-Oxadiazole, 1,2,5-oxadiazole, and 1,3,4-oxadiazole are known, but the 1,2,3-isomer is unbalanced and reverts to the diazoketone tautomer. The various oxadiazoles moiety involved in variety of drugs including Raltegravir, Butalamine, Fasiplon, Oxolamine, Pleconaril.



PHYSICAL PROPERTIES:

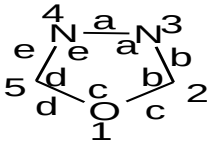


Table No. 4.1 BOND ANGLE OF OXADIAZOLES

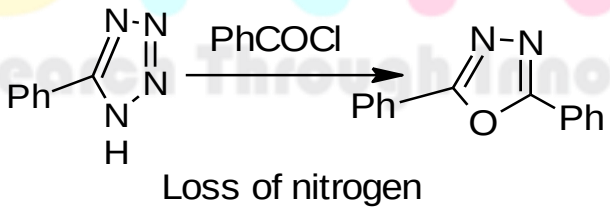
ANGLE	BOND ANGLE (^{0})
A	105.6
B	113.4
C	102.0
D	113.4
E	105.6

Table No. 4.2: BOND LENTH OF OXADIAZOLES:

BOND	BOND LENGTH (pm)
A	139.7
B	129.9
C	134.8
D	134.8
E	139.7

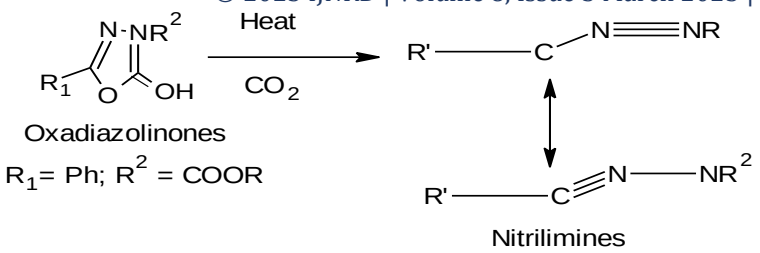
CHEMICAL PROPERTIES [11]

1. 1, 3, 4-oxadiazole are much more easily hydrolyzed by acid or alkali than 1, 2, 4 isomers.
2. **Loss of Nitrogen:** Tetrazoles with acid chlorides (in C5H5N at 50°c) give 1, 3, 4-oxadiazole.



3. **Thermal and photo-chemical reactions (Thermal reaction)**

   1, 3, 4-oxadiazole is thermally stable and this stability is increased on substitution, particularly by aryl and perfluro alkyl groups. Oxadiazolinones lose carbon dioxide at high temperature to give nitrelimines. Recyclization in the nitrelimines, formed at 210-230°C from oxadiazolinone yields 2- alkoxy-1, 3, 4-oxadiazole. [36]



4. Reactivity of 1, 3, 4-oxadiazole:

As 1 ,3, 4-oxadiazole have a relatively low electron density at carbon (positions 2 and 5) and a relatively high electron density at nitrogen (positions 3 and 4), the major reactions are nucleophilic attack at carbon, generally followed by ring cleavage and electrophilic attack at nitrogen. This reactivity towards nucleophiles, also catalyzed by acid, causes difficulties when carrying out reactions, which involve basic or acidic conditions. This ring is more stable when substituted by one or more aryl groups. Tautomeric oxadiazole react with electrophile at ring nitrogen at the exocyclic heteroatom or at both centers. Reactions in the substituent groups of alkyl or aryl 1,3,4-oxadiazole are possible but they are limited by the sensitivity of the ring to the reagent used substituent groups of alkyl or aryl 1,3,4-oxadiazole are possible but they are limited by the sensitivity of the ring to the reagent used.[12]

BIOLOGICAL SCREENING

ANTIBACTERIAL ACTIVITY [13,14]

a) Method: Cup-plate agar diffusion method using Nutrient agar.

In aradial or 2D technique, petri dishes of agar are prepared by pouring melted agar media previously inoculated with selected microorganism. After the solidification of agar cups are made with the help of borer and cups are filled with solution of suitable concentration of sample and standard respectively and are inoculated at 37°C for 24 hours. The anti-microbial agents diffuses through the agar around its cup and produces a characteristic zone of inhibition of the microorganism sensitive to the sample, the diameter of which can be measured.

b) Materials Used:

1) **Culture:** TwoG +ve and one G -ve was chosen for screening Gram positive organisms: Staphylococcus aureus(ATCC 29737) Gram negative organism: Escherichia coli (NCTC 10418)

2) **Apparatus:** Sterile petri plates, sterile cotton swabs, sterile cork borer, sterile test tubes, 1ml syringes, micropipette, inoculating loop and spirit lamp

3) **Media:** Nutrient agar media from Hi-media was used with composition:

Sr. No.	Composition	Quantity
1	Peptic digest of animal tissue	5.00 gm/lt.
2	Sodium chloride	5.00 gm/lt.
3	Beef extract	1.50 gm/lt
4	Yeast extract	1.50 gm/lt.
5	Agar	15.00 gm/lt

The pH was adjusted to 7.4±0.1 at 25^{0}C temperature.

Dissolve 28gm of media in 1000ml of distilled water by heating, sterilized by autoclaving at 121^{o}C temperature and 15 Ib/Inch^2 pressure for 15 minutes.

c) Preparation of Inoculums:

One day prior to these testing, inoculations of the above bacterial cultures were made in the Nutrient agar media and incubated at 37□C for 18-24 hrs.

d) Preparation of test solutions:

Each test compound (20 mg) was dissolved in DMSO (20 mL) to give stock solution of concentration 1000 µg/mL. Then 0.1 mL of this solution was used for testing.

e) Preparation of standard solution:

Standard drug Ciprofloxacin was used at the concentration of 100µg/mL.

f) Method of testing:

Nutrient agar plates were prepared by pouring 15-20 mL of the medium into each sterilized petri dish and were allowed to set at room temperature. The cell suspension was standardized to the density of 530 nm using spectrophotometer and was inoculated over the surface of agar medium using sterile cotton swab. The cups were scooped in each plate using a sterile cork borer of 6 mm diameter.

Then the solutions of test compounds (0.10 mL) were added in cups by using micropipettes and these plates were incubated at 37□C for 48 hrs. The zone of inhibition was measured in mm for each organism.

g) Observation:

Plates were observed within 20 to 24 hours and may be continued to incubate for 48 hours. Zone of inhibition of the compound were measured and compared with the standard compound.

Table no: 7.1 Antibacterial of synthesized compounds ((Scheme-I)

Compd.	Zone of inhibition at 200µcg/mL (in mm.)				
	E. coli	B. Subtilis	S. aureus	A. niger	C. albicans
A_1	24	**25**	**26**	15	**22**
A_2	20	23	**25**	16	21
A_3	20	24	**25**	19	**22**
A_4	**25**	26	23	20	21
A_5	24	23	**26**	**21**	**22**
A_6	20	22	24	18	**23**
A_7	21	23	22	20	21
A_8	22	24	**25**	20	**22**
A_9	23	22	20	18	**22**

Ciprofloxacin	**26**	**25**	**26**	**-**	**-**
Amphotericin B	**-**	**-**	**-**	**22**	**23**

PHARMACOLOGICAL SCREENING

ANTI- INFLAMMATORY ACTIVITY [15,16]

In-vitro anti-inflammatory activity

Carrageenan Induced hind Paw Edema:

Anti-inflammatory activity was determined by Carrageenan Induced Rat hind Paw method of winter et al. wistar rats (120-150 g) was used for the experiment. The conventional laboratory diet was fed with adequate supply of drinking water. The animals were randomly selected, marked to permit individual identification and kept in polypropylene cages for one week prior to dosing to allow acclimatization of them to laboratory conditions. The drugs were prepared as a suspension by triturating with water and 0.5% sodium CMC. The standard group received 50mg/kg body weight of Ibuprofen, test group received 200mg/kg body weight of synthesized compounds and the control group received 1% w/v of CMC.

% of Inhibition = 100 X [1- Vt / Vc]

Where,

Vt = Mean absorbance of test sample.

Vc = Mean absorbance of control

RESULT AND DISCUSSION

The synthesized compounds were subjected to various anti-bacterial, anti-fungal anti-tubercular and anti-inflammatory activities by using standard methods.

Anti-bacterial activity:

The compounds A_2, A_3, A_5, A_8, has excellent Antibacterial activity against *S. aureus*, the compound A_1, have shown Antibacterial activity against *B. subtilis*, while A_4 show Antibacterial activity against *E.coli*., when compared with standard ciprofloxacin

Fig. No. 9.1: Anti bacterial activity of synthesized compounds (Scheme I)

Anti-inflammatory activity:

All the compounds were evaluated for Anti-inflammatory activity by Carrageenan Induced Rat hind Paw method. The synthesized compounds showed better anti-inflammatory activity found comparable with standard drug zaltoprofen.

CONCLUSION

1. The present research work is a bonafide novel for the synthesis of oxadiazole.
2. The extensive literature review suggests the utilization of these heterocycles as a lead in treatment of wide variety of diseases and disorders.
3. The method of synthesis of these heterocycles starting from different substrate had been established.
4. Around nine newer derivatives of afore mentioned heterocycles were synthesized.
5. The purity of synthesized compounds was checked with the help of TLC.
6. The physical constants (Melting point) of the synthesized compounds were determined using open capillary method.
7. The structures of the synthesized compounds were established by using IR, ^{1}H NMR, and CHN analysis.
8. The synthesized compounds were screened for their anti-inflammatory and antibacterial activities.
9. Some of the compounds shows significant biological activity which can be explore as drug candidate in future.
10. The proposed work has given out many active Antibacterial and Anti- inflammatory agents. Some of the compounds have showed excellent activities. These compounds with suitable modification can be explored better for their therapeutic activities in the future.

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