SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION OF 2, 5 DISUBSTITUTED 1, 3, 4-OXADIAZOLE DERIVATIVES

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ABSTRACT:

A epic series of 2-Amidino-5-aryl-1,3,4-oxadiazole derivatives (N1-N4) have been successfully synthesized. A blend of 2-Amino-5-aryl-1,3,4-oxadiazole, powdered anhydrous aluminium chloride and acetonitrile warmed in an oil bath keeping the temperature at 150-160°C for 2 hours to synthesize the derivatives (N_1-N_4).). The structures of recently synthesized compounds were set up on the basis of FT- IR, ^{1}H NMR and Mass spectroscopic techniques. The In-vitro Anti-bacterial activity and In-vivo Anti- inflammatory activity were evaluated by recently synthesized compounds. The Evaluation of In-vitro Anti-bacterial activity was by Disc Diffusion technique used against the standard drug as Ofloxacin . Carageenan Induced Rat Paw Oedema method was used against standard drug as Ibuprofen to determine the in-vivo anti-inflammatory activity. Compounds N_1 and N_2 exhibited good antibacterial activity, compound N_4 showed moderate anti-bacterial activity and compound N_3 showed poor antibacterial activity. Compound N_4 showed good anti- inflammatory activity, compounds N_1 and N_3 showed moderate anti-inflammatory activity and compound N_2 showed poor anti-inflammatory activity.

KEYWORDS: 1, 3, 4-Oxadiazole, Antibacterial activity, Anti-inflammatory activity, Analgesic Activity

INTRODUCTION:

Heterocyclic compound may be defined as those cyclic structure in which ring contain one or more non-identical kinds of atoms. The most common types contain largely carbon atoms. Nitrogen, oxygen and sulphur are the most common heteroatoms, but many other elements, including even bromine, can also serve[1]. Oxadiazole may be defined as a five membered ring cyclic compound which contain one oxygen & two nitrogen atoms. There can be 4 (four) possible isomers of oxadiazole which will depending on location of the nitrogen atom in ring. The 1, 3, 4-oxadiazole nucleus have capacity that it can undergo different types of chemical reactions, which includes electrophillic substitution, nucleophilic substitution, thermal and photochemical reactions. This makes it a medicinal backbone on which a number of potential molecules can be prepared2. From the literature survey 1,3,4-oxadiazole nucleus has been found to possess diverse pharmacological activities such as antibacterial, anti-inflammatory, analgesic, 1,3,4-Oxadiazoles are biologically active, synthetically useful and important heterocyclic compounds, for these reasons the chemistry of 1,3,4 Oxadiazoles have been the subject of many investigations. In recent report Cerric ammonium mediated synthesis of 1,3,4-Oxadiazoles has also been described7. Derivatives of oxadiazole are used in the market such as (Tiodazosin, Nosapidil, Furamizole), in the preparation of dyes, liquid crystals and scintillators etc8.

MATERIALS AND METHODS:

General Procedure for the Synthesis of Acid chloride (A): A mixture of aromatic acid (0.01 mol), phosphorous pentachloride (0.01 mol) and benzene (5 ml) was taken in round bottom flask fitted with reflux condenser with calcium chloride guard tube and was heated on a water bath with vigorous shaking for 45 minutes at 50 °C. After the completion of reaction the content was cooled and excess of antitubercular3, anthelmintic4, anticonvulsant, muscle $POCl_3$ distilled out from the mixture to get acid chloride (A).

General Procedure for the Synthesis of 2-Amino-5-aryl- 1,3,4-oxadiazoles (B):

A mixture of acid chloride (A) and semi-carbazide (0.01 mol.) was heated on a water bath for 4-5 hours. After the completion of reaction, the unreacted acid chloride was discarded from the round bottom flask and solid residue was neutralized with aq. $NaHCO_3$ solution. Then the content was filtered to get the solid product and then it was washed with water to obtain 2-Amino-5-aryl-1,3,4- oxadiazole (B). The product was dried well and recrystallized from methanol. The progress of the reaction was checked by TLC.

General Procedure for the Synthesis of 2-Amidino-5- aryl-1,3,4-oxadiazoles (N1-N4):

A mixture of compound B (0.01 mol), powdered anhydrous aluminium chloride (4 g) and acetonitrile (41 ml) was heated on a heating mantle keeping the temperature of the mixture at 150-160 °C for 2 hours. The content was cooled and the product was decomposed using ice cold HCl. The residue was basified with aq. NH_3 solution and filtered and dried well. After then product was recrystallised from DMF. The progress of the reaction was checked by TLC.

Compound N1: 2-Amidino-5-(4-chlorophenyl)-1,3,4- oxadiazole:

Yield: 72%, mp: 150-152°C: FT-IR (cm^{-1}): 3117.11 (-NH),1671.02 (-CN), 777.44 (-CH) : 1H NMR (DMSO-d_6 , δ, ppm): 3.41 (s,1H,J= 3.413), 2.88(M,4H,J= 2.899), 2.74 (d,2H,J= 2.735), 2.48(m.1H,J= 2.493) : Mass m/z (%) : Anal. Calculated for $C_{10}H_9ON_4Cl$: C=50.73, H= 3.80, O=6.76, N= 23.67, Cl= 15.01; Found: C=50.23, H=3.76, O=6.69, N=23.44, Cl=14.85.

Compound N2: 2-Amidino-5-(3-nitrophenyl)-1,3,4- oxadiazole:

Yield: 77%, mp: 148-15 °C: FT-IR (cm^{-1}): 3121.61 (-NH),1655.75 (C=N), 713.11 (-CH) : 1H NMR (DMSO-d_6, δ, ppm) : 8.90 (s.1H,J= 8.909), 8.78 (m,4H,J= 8.773), 8.44 (d,2H,J= 8.449), 7.98 (s,1H,J= 7.982): Mass m/z (%): Anal. Calculated for $C_{10}H_9O_3N_5$: C=48.58, H=3.64, O= 19.43, N=28.34 : Found : C= 49.34, H= 3.70, O= 19.73, N=28.78.

Compound N3: 2-Amidino-5-(4-nitrophenyl)-1,3,4- oxadiazole:

Yield: 70%, mp: 142-145°C: FT-IR (cm^{-1}): 3175.58(-NH),1652.39 (C=N), 760.55(-CH) : 1H NMR (DMSO-d_6, δ,ppm) : 10.55 (s,1H,J=10.469), 10.10(M,4H,J=10.084), 8.05 (d,2H,J=8.001),: Mass m/z (%): Anal. Calculated for $C_{10}H_{11}ON_5$: C=55.29, H=5.06, O=7.37, N=32.25 : Found : C=55.76, H=5.11, O=7.43, N=32.52.

Compound N4: 2-Amidino-5-(4-aminophenyl)-1,3,4- oxadiazole:

Yield: 70%, mp: 142-145°C: FT-IR (cm^{-1}): 3175.58(-NH),1652.39 (C=N), 760.55(-CH) : 1H NMR (DMSO-d_6, δ, ppm) : 10.55 (s,1H,J=10.469), 10.10(M,4H,J=10.084), 8.05(d,2H,J=8.001),: Mass m/z (%): Anal. Calculated for $C_{10}H_{11}ON_5$: C=55.29, H=5.06, O=7.37, N=32.25 : Found :C=55.76, H=5.11, O=7.43, N=32.52.

ANTIBACTERIAL ACTIVITY:

Antibacterial activity of prepared derivatives (N_1-N_4) was evaluated by Disc Diffusion method. All the derivatives were screened for in vitro antibacterial activity against gram positive bacterial strains such as Staphylococcus aureus and Bacillus subtilis and gram negative bacterial strains such as Escherichia coli and Pseudomonas aureginosa at a concentration of 50µg/dish by using DMSO as solvent control and nutrient agar was employed as culture media. After 24 hours of incubation at 37 °C, the zone of inhibition was measured in mm. The activity was compared with standard drug Ofloxacine at a concentration of 25µ g/ml. The antibacterial activity of prepared derivatives was determined as percent of inhibition.

ANTI-INFLAMMATORY ACTIVITY:

The in vivo Anti-inflammatory activity of the synthesized compounds (N_1-N_4) was evaluated by the Carageenan Induced Rat Paw Oedema method. The compounds were tested at an oral dose of 60 mg/kg of body weight and were compared with the standard drug Ibuprofen at a dose of 40mg/kg after 3^{rd} and 6^{th} hour of inflammation induction by carageenan treatment. The percentage of inhibition for each group after 3^{rd} and 6^{th} hour was calculated and the data are expressed.

RESULTS AND DISCUSSION:

Chemistry:

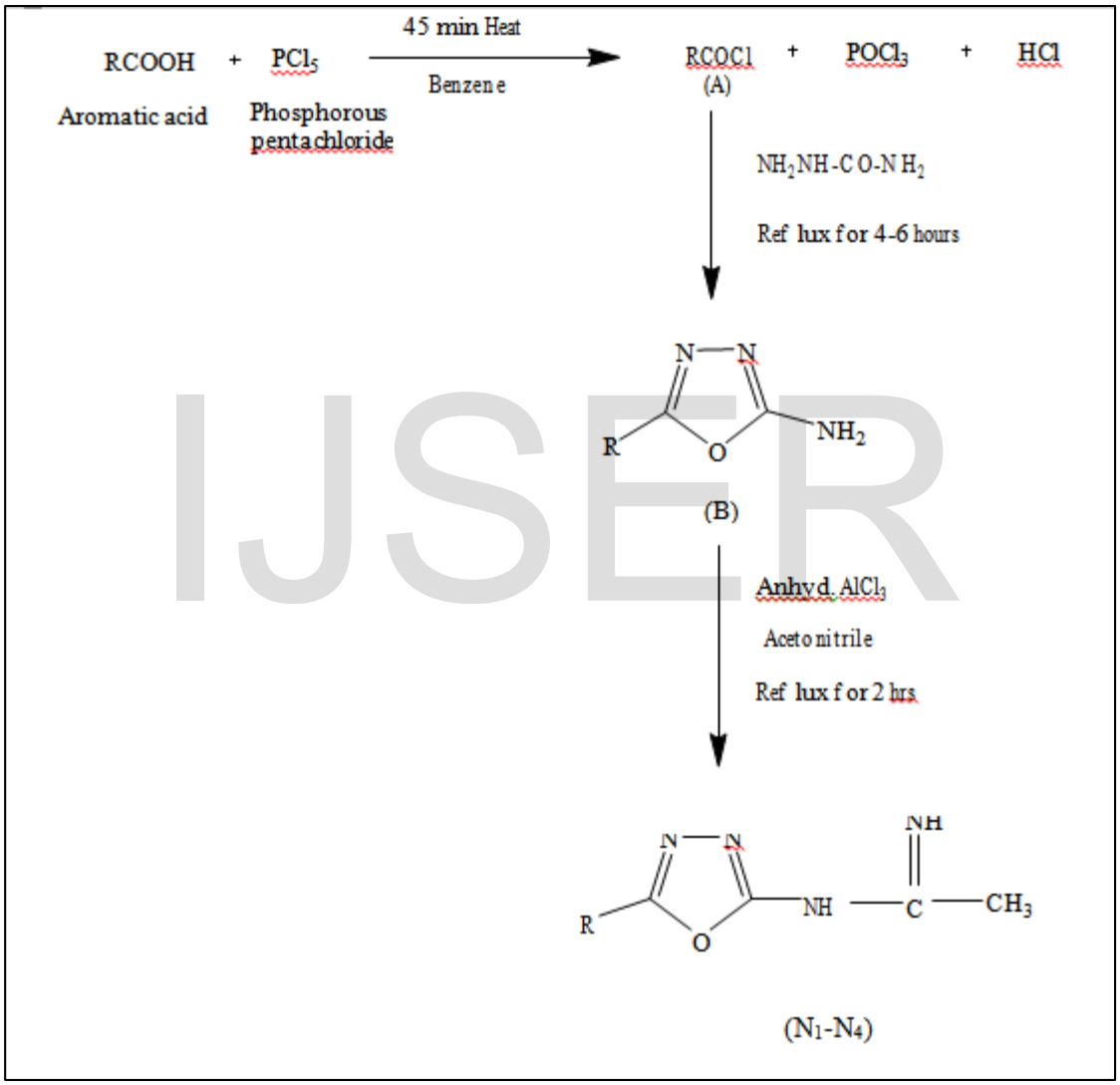
After the completion of project, it was found that the synthesized compounds (N1-N4) were obtained in good yields and in a high state of purity. The synthesized compounds 2-Amidino-5-(4-chlorophenyl)-1,3,4- oxadiazole (N_1), 2-Amidino-5-(3-nitrophenyl)-1,3,4- oxadiazole (N_2), 2-Amidino-5-(4-nitrophenyl)-1,3,4- oxadiazole (N_3) and 2-Amidino-5-(4-aminophenyl)-1,3,4-oxadiazole (N_4) were identified and characterized by IR, H NMR and Mass spectral data.

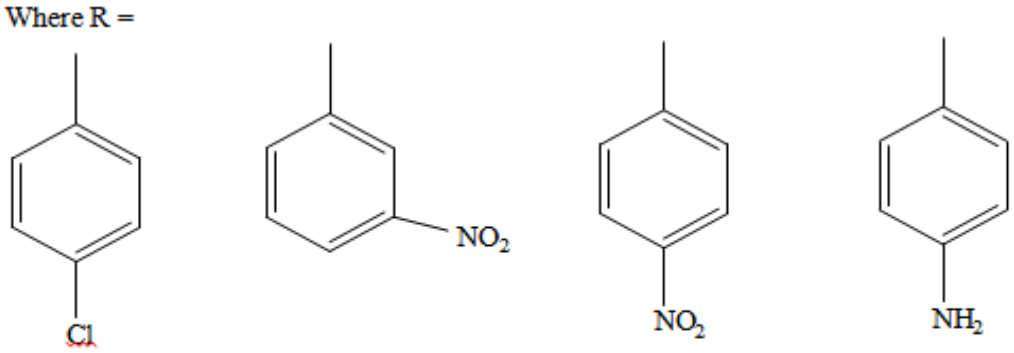
The progress of reaction was confirmed by TLC (table-1).

Table 1: Physical data of synthesized compounds (N_1-N_4)

Compounds	-R	Yield (%)	M.P. (°C)	Rf value	Molecular formula	Molecular weight
N_1	p-ClC_6H_5	72	150-152	0.6	$C_{10}H_9ON_4Cl$	236.5
N_2	m-$NO_2C_6H_5$	77	148-150	0.4	$C_{10}H_9O_3N_5$	247.0
N_3	p-$NO_2C_6H_5$	75	146-149	0.8	$C_{10}H_9O_3N_5$	247.0
N_4	p-$NH_2C_6H_5$	70	142-145	0.5	$C_{10}H_{11}ON_5$	217.0

SCHEME-1:





(N1) (N2) (N3) (N4)

ANTIBACTERIAL ACTIVITY:

All the prepared derivatives were evaluated for their antibacterial activity by Disc Diffusion method. All the compounds showed antibacterial activity in the range of 68.75-94.11% as compared with standard drug ofloxacine which completely inhibited test microorganisms. Compounds N_1 and N_2 exhibited good activity, compound N_4 showed moderate activity and compound N_3 showed poor activity (Table-2).

Table 2: **Antibacterial activity of Synthesized Compounds by Disc Diffusion Method**

Zone of inhibition in mm

Compounds	S.aureus	% of inhibition	B.subtilis	% of inhibition	E.coli	% of inhibition	P. aeruginosa	% of inhibition
N_1	14	87.50	14	82.35	14	87.50	16	88.89
N_2	12	75.00	13	76.47	13	81.25	14	77.78
N_3	13	81.25	14	82.35	15	93.75	16	88.89
N_4	11	68.75	16	94.11	14	87.50	15	83.33
Ofloxacine	16	100.00	17	100.00	16	100.00	18	100.00

ANTI-INFLAMMATORY ACTIVITY:

All the prepared derivatives were evaluated for their anti- inflammatory activity by Carageenan Induced Rat Paw Oedema method. All the compounds showed anti- inflammatory activity in the range of 31.46-35.87% after 3 hours of Carageenan treatment and in the range of 61.16-65.12% after 6 hours of Carageenan treatment when compared with Ibuprofen which completely inhibited oedema after Carageenan treatment. Compound N_4 showed good activity, compound N_1 and N_3 showed moderate activity and compound N_2 showed poor activity (table-3).

Table 3: Anti-inflammatory activity of synthesized compounds by Carageenan Induced Rat Paw Oedema Method

Compounds	Dose mg/kg	Inhibition of paw oedema after 3 hrs (%)	Inhibition of paw oedema after 6 hrs (%)
N_1	60	35.87	63.80
N_2	60	31.46	61.16
N_3	60	35.86	63.81
N_4	60	35.38	65.12
Control	-	-	-
Ibuprofen	40	40.29	66.44

ANALGESIC ACTIVITY:

The activity was carried out on wistar mice weigh between 20-30gm using tail flick method. The test compound and standard drug (pentazocin 5mg/kg) were administered orally.

Table 4: Analgesic activity of synthesized compounds by wistar mice flick Method

Compounds	Dose mg/kg	Inhibition of paw oedema after 3 hrs (%)
N_1	5	58.85
N_2	5	88.85
N_3	5	76.32
N_4	5	80.5
Control	-	-
Pentazocin	5	94.5

CONCLUSION:

In my present research work, a novel series of of 2-Amidino-5-aryl-1,3,4-oxadiazole derivatives were synthesized and were obtained in good yields. The four derivatives were isolated and purified by Thin layer Chromatography and Column Chromatography respectively.

In vitro anti-bacterial activity was evaluated by Disc Diffusion technique against Gram +ve strains such as S. aureus, B. subtilis and Gram –ve strains such as E. coli and P. aeruginosa. All the prepared derivatives were evaluated for their anti-inflammatory activity by Carageenan Induced Rat Paw Oedema method. The results revealed that compounds N1 and N2 exhibited good antibacterial activity and compound N_4 showed good anti-inflammatory

activity. Comparison of antimicrobial and anti-inflammatory screening data suggested that the compounds N_1, N_2 and N_4 were active against Gram positive bacteria & Gram negative bacteria, and compounds N_1, N_3 and N_4 were most active against paw inflammation. Compound N_2 and N_4 shows good analgesic activity while compound N_1 and N_3 shows moderate analgesic activity.

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