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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL SCREENING OF 3-(5-SUBSTITUTED PHENYL- 1, 3, 4 - OXADIAZOL- 2 - YL) PYRIDINES

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ABSTRACT

A series of 3- (5- substituted phenyl- 1, 3, 4- Oxadiazol- 2- yl) pyridines was designed, synthesized and characterized for evaluation of potential antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella species* and antifungal activity against *Candida albicans*. Structure-activity relationship led to the conculsion that compound $IV_{b,}$ and IV_e exhibited good antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* and comparatively poor activity against *Klbesiella species* and *Escherichia coli*. Compound C exhibited antibacterial activity against *Staphylococcus aureus* and very poor activity against rest of organism. Compounds IV_a, IV_b and IV_e showed poor antifungal activity against *Candida albicans* and compounds IV_c and IV_d showed no significant antifungal activity. The interesting antimicrobial profile of compound IV_b and IV_e led us to select this derivative for further development. Present research our expected compounds were given below.

INTRODUCTION: Heterocyclic ring system of Oxadiazole and their derivatives aroused great interest in the past and recent years due to their wide variety of biological properties such as Antiviral [1], antitumor [1-2], Anti-inflammatory [3], CNS [4-9], Local anesthetic [5-10], Antimicrobial activity [12] and their derivatives of Oxadiazoles are also biologically important. In view of these findings, we have attempted to incorporate both the biolabile components together to give a confine structure like title compound for evaluating its antimicrobial activity. Our efforts for the synthesis of compounds possessing antifungal[19] and antibacterial activities[19] led us to the discovery of several derivatives of Oxadiazoles. Among these amino Oxadiazole derivatives have displayed good antibacterial activity, while nitro and amino oxadiazole derivatives showed poor antifungal activities.

EXPERIMENTAL:

Chemistry: Nicotinic acid (I) on treatment with ethanol in presence of Conc. Sulphuric acid affords pyridine- 3- carboxylate (II) Compound (II) when reacted with hydrazine hydrate in ethanol yielded pyridine- 3- carboxylic acid hydrazine (III) and on treatment with aromatic acids in the presence of phosphorus oxychloride affords 3- (5- substituted phenyl-1, 3, 4- Oxadiazol- 2- yl) pyridines (IV_{a-e}). The overall reaction of Scheme given below;

Synthesis of ethyl pyridine- 3- carboxylate (II): A mixture of pure nicotinic acid (I) (0.5mol), 100ml of ethanol and 50ml of Conc. Sulphuric acid were refluxed for 4hrs on a steam bath. After cooling the solution, it was poured in to crushed ice and neutralized by strong ammonia solution, ester separates as oil. Extract the solution with anhydrous magnesium sulphate. Ether was removed by evaporation on a water bath and the residue of ethyl pyridine-3-carboxylate was obtained.

Synthesis of Pyridine- 3- Carboxylic acid Hydrazine (III): A mixture of ethyl pyridine-3-carboxylate (II) and hydrazine hydrate in equimolar portion and 15ml of ethanol was refluxed for 4-6hrs. On cooling crude product was separated out. It was filtered, collected and recrystallized from ethanol.

General Procedure for Synthesis of 3 (5- aryl- 1, 3, 4- oxadiazole- 2- yl) - pyridine derivatives (IV_{a-e}): A mixture of nicotinic acid hydrazine (III) (0.01mol) and various aromatic acids (0.01mol) in phosphorus oxychloride (5-10ml) was refluxed for 5-6hrs. The contents were cooled and poured into crushed ice. It was neutralized with sodium bicarbonate solution and resulting solid was filtered, dried and recrystallized from suitable solvent to obtained corresponding derivatives.

Synthesized Compound a: IR: (-C=N-) 1600; (-C-N-) 1357; (-C-O-) 1070; NMR and Mass: (Ar-H) 7.57-7.62 (5H, m); (Pyridyl-H) 8.16- 8.18 (4H, m); (M/Z) 223 (M^+) 223.07 (100%), 224.08 (14.2%), 225.08 (1.1%).

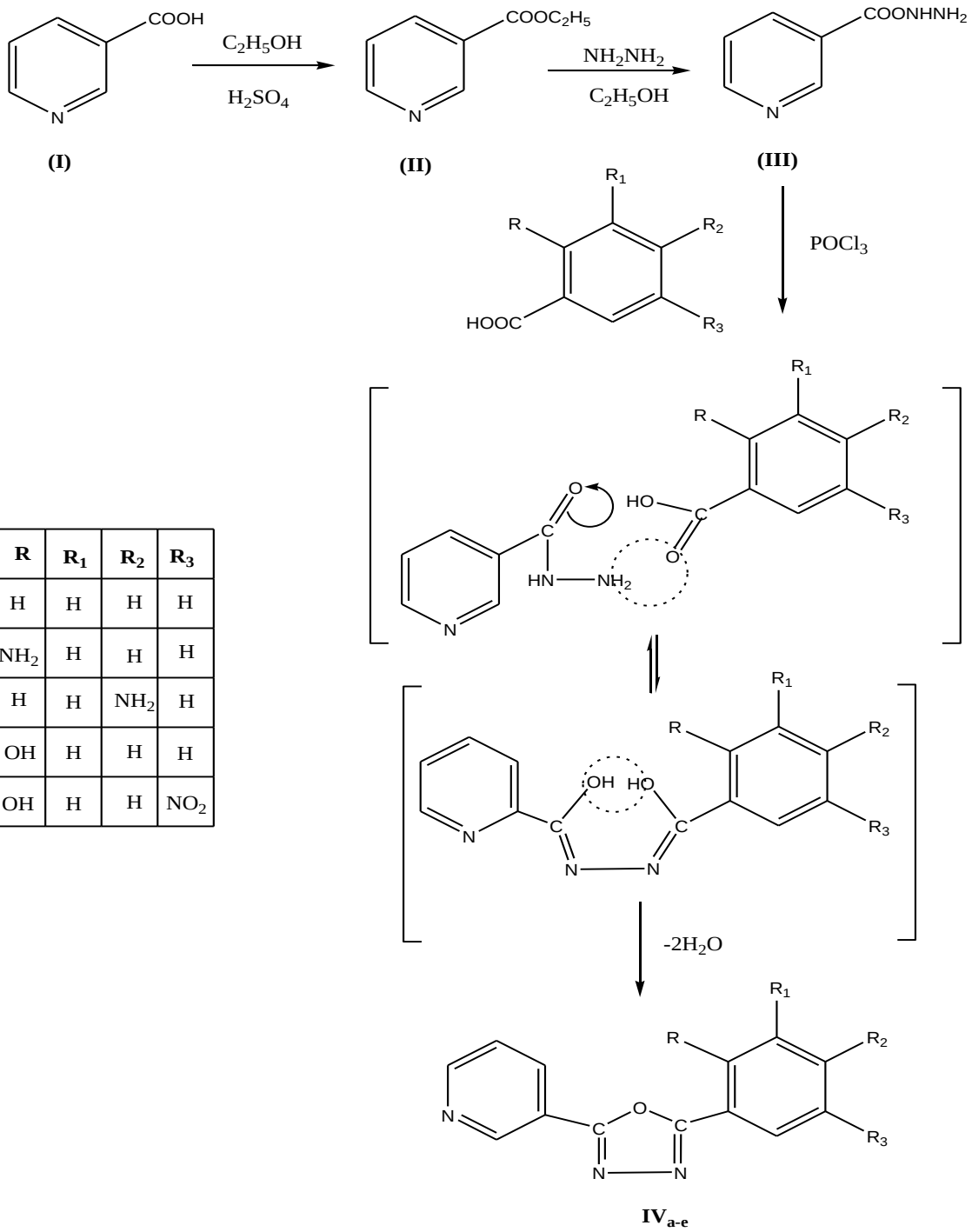
Compound b: IR: (-C=N-) 1604; (-C-N-) 1357; (-C-O-) 1145; (-NH-) 3457; NMR and Mass: (Ar-H) 7.45-7.65 (4H, m); (Pyridyl-H) 7.81-7.83 (4H, m); (-NH_2-) 4.44 (2H, t); (M/Z) 238 (M^+) 238.09 (100%), 239.09 (14.2%), 239.08 (1.5%).

Compound c: IR: (-C=N-) 1598; (-C-N-) 1344; (-C-O-) 1095 (-NH-); 3473 NMR and Mass: (Ar-H) 6.62-7.65 (4H, m); (Pyridyl-H) 8.04 9.37 (4H, m); (NH_2) 2.88 (2H, d); (M/Z) 238 (M^+) 238.09 (100%), 239.09 (14.2%), 239.08 (1.5%).

Compound d: IR: (-C=N-) 1598; (-C-N-) 1361; (-C-O-) 1108; (-OH-) 3525; NMR and Mass:(Ar-H) 7.40- 7.57 (4H, m); (Pyridyl-H) 7.90-8.33 (4H, m); (-NH_2-) 2.88 (2H, d); (-OH) (1H, s) (M/Z) 239 (M^+), 239.07 (100%), 240.07 (15.2%).

Compound e: IR: (-C=N-) 1598; (-C-N-) 1344; (-C-O-) 1095 (-NH-); 3473 NMR and Mass: (Ar-H) 6.62-7.65 (4H, m); (Pyridyl-H) 8.04-9.37 (4H, m); (-NH_2-) 2.88 (2H, d); (M/Z) 284 (M^+), 284.05 (100%), 285.06 (14.3%), 286.06 (1.8%).

SCHEME



Compounds	R	R_1	R_2	R_3
a	H	H	H	H
b	NH_2	H	H	H
c	H	H	NH_2	H
d	OH	H	H	H
e	OH	H	H	NO_2

SCHEME 1: SYNTHESIS OF TITLE COMPOUNDS

The course of reaction and the purity were ascertained by performing TLC. Melting points were determined in open capillaries and are uncorrected. IR spectra were recorded in Perkin-Elmer 297 spectrophotometer with KBr pellets and only noteworthy absorption level (reciprocal centimeter) is listed. ^{1}H-NMR spectra were recorded at 400 MHz on Bruker amx 400 MHz spectrophotometer in $CDCl_3$ using tetramethyl silane (TMS) as internal standard and ^{13}C-NMR spectra were recorded at 100 MHz on Bruker amx 400 MHz spectrophotometer in $CDCl_3$.

Mass spectra were recorded on a VG analytical 7070E instrument equipped with VG 11-250 data acquisition system. Elemental analysis (C,H and N) were carried out on a Carlo Erba Model 1106 and Perkin Elmer models 240 CHN analyzer. The results are within $\pm$0.4% of the theoretical values. Unless otherwise stated, all the starting materials and reagents were of high grade, purchased from Aldrich, Fluka and Merck. All the solvents were distilled prior to use. The structures of the synthesized compounds have been established on the basis of their analytical and spectral data. All the compounds have been screened for their antimicrobial activities.

Pharmacology:

***In vitro* Antibacterial and Antifungal Activity:** The *in vitro* activities of the compounds were tested in Sabourauds dextrose broth (SDB; Hi- media, Mumbai) for fungi and Nutrient broth (NB; Hi-media, Mumbai) for bacteria by the two fold serial dilution method [19-20]. The test compounds were dissolved in dimethylsulfoxide (DMSO) to obtain 1mg ml^{-1} stock solution. Seeded broth (broth containing microbial spores) was prepared in NB form 24 hrs old bacteria cultures on nutrient agar (Hi-media, Mumbai) at 37$\pm$1^{o}C while fungal spores from 24hr to 7 days old Sabourauds agar slant cultures were suspended in SDB. The colony forming units (cfu) of the seeded broth were determined by plating technique and adjusted in the range of 10^4-10^5 cfu ml^{-1}. The final inoculam size was 10^5 cfu ml^{-1} for antibacterial assay [19] and 1.1-1.5 X 10^2 cfu ml^{-1} for antifungal assay [19]. Testing was performed at pH 7.4$\pm$0.2. Exactly 0.2 ml of the solution of test compound was added to 1.8 ml of seeded broth to form the first dilution.

One milliliter of this was diluted with a further 1 ml of the seeded broth to give the second dilution and so on till six such dilutions were obtained. A set of assay tubes containing only seeded broth was kept as control and likewise solvents control were also run simultaneously. The tubes were incubated in BOD incubators at 37$\pm$1^{o}C for bacteria and 28$\pm$1^{o}C for fungi. The minimum inhibitory recorded by visual observations after 24hr (for bacteria) and 72-96h (for fungi) of incubation. Ampicillin and Griesofulvin were used as standards.

RESULTS AND DISCUSSION:

Antibacterial Activity [13, 15, 19, 22]: These synthesis of a series of these derivatives was carried out and the obtained compounds IVa-e were tested for their *in vitro* antibacterial activity against some Gram positive [*Staphylococcus aures* NCIM-2492 and *Bacillus subtilis* NCIM-2439] and Gram negative [*Escherichia coli* NCIM-2345 and *Klebsiella pneumoniae* species bacteria. Ampicillin used as standard drug whose minimum inhibitory concentration values are summarized in Table 1. The antibacterial screening put in evidance that all the synthesized novel 3- (5- substituted phenyl- 1, 3, 4- oxdiazol- 2- yl) pyridines IV_b and IV_e exhibited a wide spectrum of antibacterial profile in vitro against the test organisms. Compound IV_a without

any substitution, compound IV_b amino group at R-position, Compound IV_c, amino group at R_2 position, among these Compounds showed exhibited activity against *Escherichia coli* and *Klebsiella species*. Compound IV_d OH group substituted at R position and Compound IV_e, OH and NO_2 group substituted at R and R_3 position. Among these Compounds showed good activity against *Escherichia coli*. Overall Compounds IV_b and IV_e exhibited good antibacterial activity.

TABLE-1: DATA FOR MINIMUM INHIBITORY CONCENTRATION FOR ANTIBACTERIAL ACTIVITY

Compound	*Bacillus subtilis*						*Staphylococcus aureus*						*Escherichis coli*						*Klebsiella species*					
	Conc. (µg/ml)						Conc. (µg/ml)						Conc. (µg/ml)						Conc. (µg/ml)					
	1000	500	250	125	62.5	31.25	1000	500	250	125	62.5	31.25	1000	500	250	125	63	31.25	1000	500	250	125	62.5	31.25
IVa	-	-	-	+	+	+	-	-	-	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+
IVb	-	-	-	-	+	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	+	+	+	+
IVc	-	+	+	+	+	+	-	-	-	-	-	+	-	+	+	+	+	+	-	+	+	+	+	+
IVd	-	-	-	+	+	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	+	+	+
IVe	-	-	-	-	+	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	+	+	+
Control +Ve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Control -Ve	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Ampicillin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

'+' indicates presence of growth; '-' indicates presence of growth

Antifungal Activity [13, 15, 19, 22]: The in vitro antifungal activity of compounds IV_{a-e} were examined against the fungal strains Viz., *Candida albicans* (NCIM-C27), Griesofulvin was used as standard drug whose minimum inhibitory Concentration values are reproduced in Table 2.

The antibacterial profile of Compounds IV_b and IV_e were poor antifungal activities against *Candida albicans*. Compound IV_c, amino group substituted at R_2 position and compound IV_d, OH group substituted at R position were showing good antifungal activity at all concentration. Compound IV_a, IV_b and IV_e were showed moderate antifungal activity except at high concentration of 1000µg/ml. Overall Compound IV_c and IV_d were excellent antifungal activity against *Candida albicans*.

TABLE 2: DATA FOR MINIMUM INHIBITORY CONCENTRATION FOR ANTIFUNGAL ACTIVITY

Compounds	*Candida albicans*					
	Concentration (µg/ml)					
	1000	500	250	125	62.5	31.25
IVa	-	+	+	+	+	+
IVb	-	+	+	+	+	+
IVc	+	+	+	+	+	+
IVd	+	+	+	+	+	+
IVe	-	+	+	+	+	+
Control +Ve	+	+	+	+	+	+
Control -Ve	-	-	-	-	-	-
Griesofulvin	-	-	-	-	-	-

CONCULSION: A close examination of in vitro antibacterial and antifungal profile of variously substituted novel 3- (5- substituted phenyl- 1, 3, 4- Oxadiazol- 2- yl) pyridines against tested bacterial and fungal strains provide a better structure-activity correlate which is summarized above. Amino and nitro group substituted oxadiazole derivatives showed eliciting inhibition of all bacteria and poor antifungal activities of these two compounds against *Candida albicans*. All the synthesized derivatives exhibited good antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* and comparatively poor activity against *Klbesiella species* and *Escherichia coli*. Compounds IV_a, IV_b and IV_e showed poor antifungal activity against *Candida albicans* and compounds IV_c and IV_d showed no sigfinicant antifungal activity.

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