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SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL EVALUATION OF NOVEL 1-(PYRIDINE-2YL)-1H-PYRAZOLE-5-CARBOXAMIDE DERIVATIVES

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Keywords:

Pyrazole, 1-(pyridine-2yl)-1H-pyrazole-5-Carboxamide, Antibacterial activity

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ABSTRACT: Introduction: Bacterial infections are associated with significant morbidity and mortality globally. This scenario is further complicated by the development of antibiotic resistance, which has an adverse impact on the world economy by increasing the treatment cost and prolonging the hospital stay. It also requires use of reserve antibiotics which have higher toxicity. Hence, new antibacterial drugs are urgently needed to combat multidrug-resistant organisms. The present study was designed to synthesize novel 1-(pyridine-2yl)-1H-pyrazole-5-Carboxamide derivatives and to screen them for antibacterial activity. **Methods:** A simple, multistep procedure was used to synthesize novel 1-(pyridine-2yl)-1H-pyrazole-5-Carboxamide derivatives. They were characterized by LCMS and NMR. Their antibacterial activity was evaluated against four bacterial strains namely *E. coli*, *Pseudomonas aeruginosa*, *Bacillus cereus and Proteus mirabilis* by agar well diffusion method. Ciprofloxacin was used as the reference standard. **Results:** Nine novel 1-(pyridine-2yl)-1H-pyrazole-5-Carboxamide derivatives (8a-8i) were synthesized by a cost-effective procedure. The yield of the compounds was good, ranging from 73% to 85%. Compounds 8b, 8h and 8i showed moderate activity against E coli, compound 8g against Bacillus cereus and compound 8a against Proteus mirabilis, respectively. **Conclusion:** A series of novel 1-(pyridine-2yl)-1H-pyrazole-5-Carboxamide derivatives (8a-8i) were synthesized by a simple, cost-effective procedure with good yield. Some of the derivatives had moderate antibacterial activity against *E. coli, Bacillus cereus* and *Proteus mirabilis.*

INTRODUCTION: Infectious diseases, especially bacterial infections, pose a serious risk to human health and survival [1]. They are responsible for extensive morbidity and mortality globally, leading to great strain on global healthcare [2].

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Antibiotics have played an important role in the control of bacterial infections. However, due to the rampant irrational use of antibiotics, many bacterial species have developed antibiotic resistance [3].

Some of the drug-resistant bacteria which are notorious for causing extensive mortality are *Staphylococcus aureus,* resistant to methicillin and vancomycin (MRSA and VRSA), *E. coli* producing extended-spectrum β-lactamase (ESBL) and drug-resistant TB (DR-TB) [4, 5]. Treatment of infections caused by drug-resistant bacteria requires higher

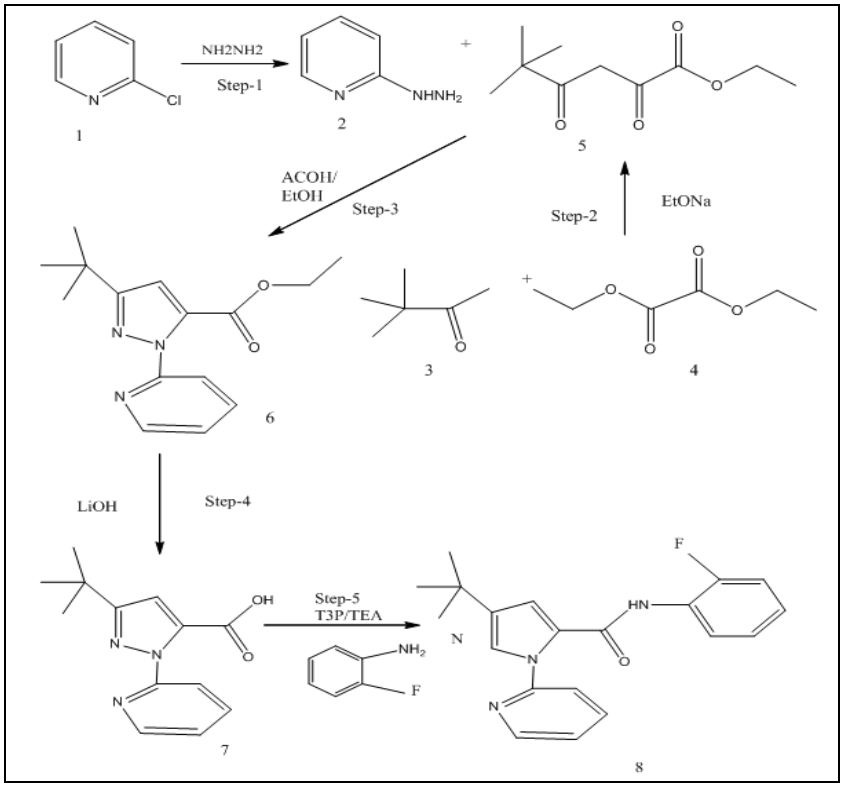
drug doses, use of reserve antibiotics with higher toxicity, and prolonged hospital admissions [6]. Hence, antibiotic resistance adversely affects global public health and economic development, necessitating an urgent search for new antibacterial drugs [7]. Pyrazoles are an important class of compounds found naturally and synthesized chemically and consist of a five-membered ring with two nitrogen and three carbon atoms [8]. Because of this unique structure, they act as a scaffold and exhibit various biological activities such as anticancer, anti-inflammatory, antifungal, analgesic, antioxidant, antidepressant, hypoglycemic *etc* [9-12]. They also serve as lead compounds for pharmaceutical development for various applications [13]. Based on the available literature and in continuation of our previous efforts to study the various activities of novel pyrazole derivatives [14], the present study was designed to synthesize novel 1 - (pyridine-2yl) - 1H-pyrazole – 5 - Carboxamide derivatives and to screen them for antibacterial activity against *E. coli*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Proteus mirabilis*.

MATERIALS AND METHODS:

Chemicals and Reagents: Toluene, dry THF and ethanol were purchased from spectrochem. The rest of the reagents were procured from Sigma-Aldrich. All reactions were conducted using silica gel under a nitrogen atmosphere.

^{1}H &^{13}C NMR and IR spectra were recorded with Bruker AMX 400 instrument and Perkin-Elmer 1600 series FTIR spectrometer, respectively. Agilent 1200 series LC & micro mass ZQ spectrometer were used to obtain LCMS data. Column chromatography was done using silica gel (230-400 Mesh).

Synthesis of 1-(pyridine-2yl)-1H-pyrazole-5-Carboxamide Derivatives: 2.03 mmol of Triethylamine, 1.066 mmol of 3-(tert-butyl)-1-(pyridine-2-yl)-1H-pyrazole-5-carboxylic acid in ethyl acetate and 1.066 mmol of corresponding amine were mixed. The resulting mixture was cooled. After adding 1.52 mmol of T_3P dropwise and stirring it at room temperature for 16 hr, the reaction mixture was diluted with 50 ml of ethyl acetate. It was washed with water and saturated brine solution and dried using anhydrous sodium sulfate. Reduced pressure was used to concentrate the mixture and column chromatography to purify the crude mixture. (Scheme 1) LCMS and NMR data confirmed the characteristics of the novel compounds (8a-8i).



SCHEME 1: SYNTHESIS OF 1-(PYRIDINE-2YL)-1H-PYRAZOLE-5- CARBOXAMIDE DERIVATIVES

Pharmacological Activity:

Ethical Approval: Institutional ethics committee approved the study protocol.

Antibacterial Activity: The antibacterial activity of the newly synthesized compounds was determined against the following bacterial strains - *E. coli, Pseudomonas aeruginosa, Bacillus cereus* and *Proteus mirabilis* by agar well diffusion method.

Preparation of Inoculums for Antibacterial Activity: Stock cultures of bacterial strains were maintained on nutrient agar slopes at 4°C. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton Broth (MBH) at 25°C. The cultures were diluted with fresh Mueller-Hinton broth to achieve optical densities corresponding to 2 x 10^6 colony-forming units (CFU/ml).

Agar well Diffusion Method: The novel synthetic compounds (8a-8i) were screened for antibacterial activity and compared with ciprofloxacin (100 µg/ml) *in-vitro* by agar well diffusion method. Lawn culture was prepared by spreading a volume of the bacterial inoculum over the Muller Hinton agar surface. Wells were punched in these plates aseptically using a well cutter at the required distance and 30µl of various test compounds was added in to the well using sterilized micropipettes. These plates were incubated overnight at 37°C. Control experiments were conducted under similar condition with ciprofloxacin as the standard antibacterial agent. The antibacterial activity of the test compounds was determined by measuring the size of the zone of growth inhibition after 18-24 hrs at 37°C. Each experiment was replicated three times and the mean of three determinations was considered. Zone of inhibition measuring <8 mm was considered as inactive against the bacteria [15, 16].

RESULTS:

Synthesis and Yield of Pyrazole Derivatives: Nine novel 1-(pyridine-2yl)-1H-pyrazole-5-Carboxamide derivatives (8a-8i) were prepared using **Scheme 1**. The yields of these derivatives are presented in **Table 1**.

Antibacterial Activity: The antibacterial activity of the synthesized pyrazole derivatives (8a-8i) was evaluated against four pathogenic bacterial strains by agar well diffusion method. Among these strains, three were gram-negative - *E. coli*, *Pseudomonas aeruginosa, Proteus mirabilis* and one was Gram-positive – *Bacillus cereus*. Ciprofloxacin was the reference standard used for comparison. The antibacterial activity of pyrazole derivative was measured as a zone of growth inhibition in mm. **Table 2** Zone of inhibition measuring <8 mm was considered as inactive against the bacteria.

Among the synthesized compounds, compounds 8b, 8h and 8i showed moderate activity against *E. coli*, compound 8g against *Bacillus cereus* and compound 8a against Proteus mirabilis, respectively **Table 2.**

TABLE 1: YIELD OF VARIOUS NOVEL 1 -(PYRIDINE - 2YL) - 1H – PYRAZOLE – 5 - CARBOXAMIDE DERIVATIVES

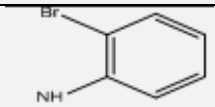
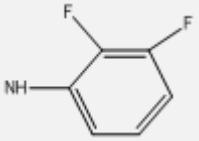
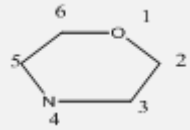
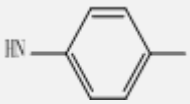
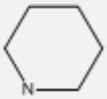
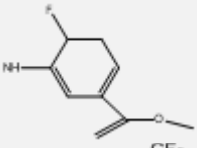
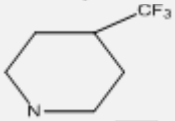
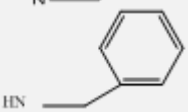
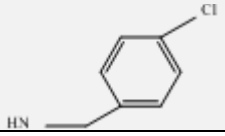
Compounds	Structure	Yield
8a		82%
8b		79%
8c		83%
8d		75%
8e		80%
8f		73%
8g		85%
8h		78%
8i		81%

TABLE 2: ANTIBACTERIAL ACTIVITY OF THE 1-(PYRIDINE-2YL)-1H-PYRAZOLE-5- CARBOXAMIDE DERIVATIVES

Compound No.	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bacillus cereus*	*Proteus mirabilis*
8a	NZ	4	5	9
8b	11	NZ	NZ	6
8c	5	NZ	4	7
8d	NZ	6	3	NZ
8e	4	8	NZ	NZ
8f	9	NZ	6	NZ
8g	NZ	NZ	11	6
8h	12	NZ	6	8
8i	11	4	NZ	7
Ciprofloxacin	15	16	15	9

Zone of growth inhibition in mm. Values are mean of three determinations, the ranges of which are less than 5% of mean in all cases. NZ: No zone of growth inhibition.

DISCUSSION: Bacterial infections caused by drug-resistant organisms cause significant morbidity and mortality and adversely affect the global economy [17]. Since, the rate of resistance development is faster than the development of new antibiotics, there is an urgent need to develop novel antibacterial drugs [18]. In the present study, we have synthesized novel 1-(pyridine-2yl)-1H-pyrazole-5-Carboxamide derivatives and screened them for antibacterial activity against *E. coli, Pseudomonas aeruginosa, Bacillus cereus* and *Proteus mirabilis*.

Nine novel 1-(pyridine-2yl) - 1H – pyrazole – 5 - Carboxamide derivatives (8a-8i) were synthesized by a cost-effective procedure with good yield. Their antibacterial activity was evaluated against four bacterial strains, namely *E. coli*, *Pseudomonas aeruginosa, Bacillus cereus* and *Proteus mirabilis* by agar well diffusion method. Compounds 8b, 8h and 8i showed moderate activity against *E. coli*, compound 8g against *Bacillus cereus* and compound 8a against proteus mirabilis respectively. None of the novel compounds were effective against *Pseudomonas aeruginosa*. The antibacterial activity of pyrazole derivatives against *E. coli* and *Bacillus cereus* has been reported by various studies [19-22].

Hence, the antibacterial activity of the novel compounds (8a-8i) can be attributed to the pyrazole moiety. The difference in the antibacterial activity of the novel pyrazole compounds (8a-8i) against the tested bacterial strains can be explained by the differences in the cell wall structure of gram positive and negative organisms and the structures of the novel pyrazole derivatives themselves. According to a recent report, the antibacterial activity of pyrazole derivatives is due to their action on different metabolic pathways in gram-positive and gram-negative bacteria [23].

CONCLUSION: A series of novel 1-(pyridine-2yl)-1H-pyrazole-5-Carboxamide derivatives (8a-8i) were synthesized by a simple, cost-effective procedure with good yield. Some of these derivatives have moderate activity against *E. coli*, *Bacillus cereus* and *Proteus mirabilis*.

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ETHICAL APPROVAL: The study was approved by the institutional ethics committee.

SOURCE OF SUPPORT: Nil

CONFLICTS OF INTEREST: None declared

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