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PYRAZOLINE DERIVATIVES: SYNTHESIS AND ANTIBACTERIAL STUDIES

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Abstract- Ten pyrazoline derivatives were synthesized and their antibacterial activity was studied against four Gram positive *Bacillus cereus* ATCC11778, Staphylococcus *aureus* ATCC29737, *Staphylococcus epidermidids* NCIM2493 and *Micrococcus luteus* ATCC10240, and three Gram negative bacteria viz. *Proteus mirabilis* NCIM2241, *Escherichia coli* ATCC25922 and *Klebsiella aerogenes* NICM2098 bacteria. The antibacterial activity was done using Agar well diffusion method. The pyrozoline derivatives showed different activity against different bacterial strains depending on their structural formula. Gram positive bacteria were more susceptible than Gram negative bacteria. *E. coli* was the most resistant bacteria and *B. cereus* was the most susceptible bacteria. The pyrazoline derivatives which had nitro group at para position showed best antibacterial activity.
Keywords- Pyrazoline derivatives, antibacterial activity, Gram positive bacteria, Gram negative bacteria.

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Introduction

In light of the recent emergence of bacteria, which are resistant to multiple antimicrobial drugs posing a challenge for the treatment of infections, the need to discover new antimicrobial substances for use in combating such micro-organisms become pertinent. Resistant bacteria represents a challenge in the treatment of infections, which are well known, necessitated the need to find new substances with antimicrobial properties to be used in the combat to these micro organisms.

Pyrazolines are reported to be good therapeutic agents. They are known to have various activities [1-5] viz. antidiabetic, tranquilizer, hypoglycemic, diuretic, anticonvulsant, cardiovascular, herbicidal, anti inflammatory, bactericidal, insecticidal, fungicidal, analgesic, anti microbial etc. Abid and Azam reported anti-amoebic activity of some substituted cyclic pyrazolines [6]. The antitumor activity of some pyrazolines have also been reported by Al-Saadi and Kucukguzel, et al [7,8]. The anti inflamnmatory and ulcerogenic and antidepressant activity have also been found for these compounds [9,10]. Ahn, et al have synthesized cyano pyrazoline derivatives and documented them as dipeptidyl peptidase(DP)-IVinhibitors and antidiabitic agents [11]. Ucar, et al have synthesized 1-N-substituted thiocarbamoyl-3-phenyl-5-thienyl-2-pyrazolines and reported as a novel cholinesterase and selective monoamine oxidase B inhibitors for the treatment of Parkinson's and Alzeimer's disease [12]. Recently, some workers studied the anti amoebic activity and anti androgenic activity of a series of new substituted pyrazoline derivatives [13,14]. These biological properties prompted us to synthesize some pyrazoline derivatives which are reported in this paper along with their characterization, and finally their potential as antibacterial agents.

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Experimental

All chemicals used in this investigation were reagent grade and were purified when necessary.

Synthesis: The compounds investigated in the present study were pyrazoline derivatives which are given below:

- 2-chloro-6-fluoro-3-[3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-5-yl]quinoline
- 2-chloro-6-fluoro-3-[3-(3-nitrophenyl)-4,5-dihydro-1*H*-pyrazol-5-yl]quinoline
- 2-[5-(2-chloro-6-fluoroquinolin-3-yl)-4,5-dihydro-1*H*-pyrazol-3-yl]phenol
- 4-[5-(2-chloro-6-fluoroquinolin-3-yl)-4,5-dihydro-1*H*-pyrazol-3-yl]aniline
- 2-chloro-6-fluoro-3-[3-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazol-5-yl]quinoline
- 3-[5-(2-chloro-6-fluoroquinolin-3-yl)-4,5-dihydro-1*H*-pyrazol-3-yl]aniline
- 2-chloro-6-fluoro-3-[3-(4-methylphenyl)-4,5-dihydro-1*H*-pyrazol-5-yl]quinoline
- 4-[5-(2-chloro-6-fluoroquinolin-3-yl)-4,5-dihydro-1*H*-pyrazol-3-yl]phenol
- 2-chloro-3-[3-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-5-yl]-6-fluoroquinoline
- 3-[3-(4-bromophenyl)-4,5-dihydro-1*H*-pyrazol-5-yl]-2-chloro-6-fluoroquinoline.

All these compounds were synthesized according to reaction scheme 1. The physical constants such as molecular formula, molecular weight, melting point, % yields, and R_f values along with the solvent system of all these pyrazoline derivatives are given in Table 1.

The IR spectra (KBr pellets) were scanned on IR (SHIMADZU-FTIR-8400) over the frequency range from 4000 – 400 cm^{-1}. ^1H NMR spectra were scanned on Bruker Spectrometer (400 MHz) by using deuterated DMSO as a solvent. The Mass spectra were scanned on GCMS-SHIMADZU-QP2010.

Antibacterial Activity

Test Microorganisms

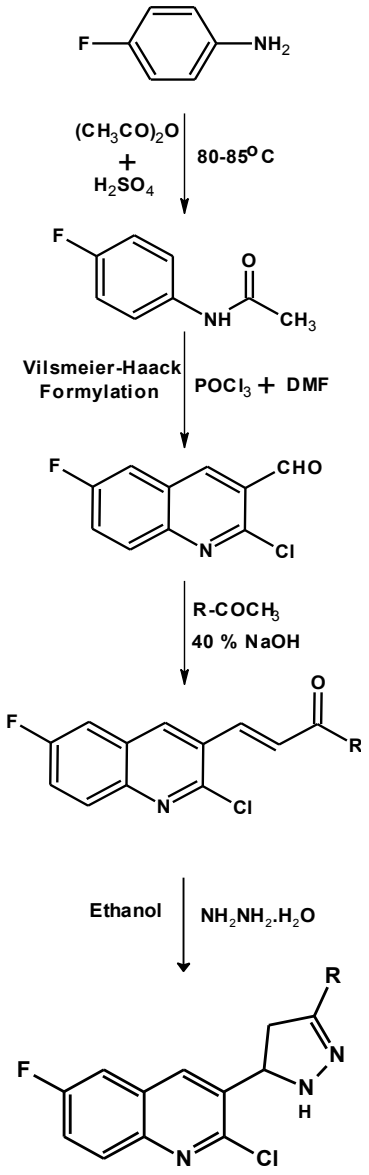
The synthesized compounds were tested for its antibacterial activity against four Gram positive *Bacillus cereus* ATCC11778, Staphylococcus *aureus* ATCC29737, *Staphylococcus epidermidids* NCIM2493 and *Micrococcus luteus* ATCC10240, and three Gram negative bacteria viz. *Proteus mirabilis* NCIM2241, *Escherichia coli* ATCC25922 and *Klebsiella aerogenes* NICM2098 bacteria. Microorganisms were obtained from National Chemical Laboratory (NCL), Pune, India. Microorganisms were maintained at 4°C on nutrient agar slants.

Preparation of the Test Compound

The synthesized test compounds were dissolved in DMSO at a concentration of 20 mg/ml. DMSO is a versatile non-aqueous dipolar aprotic solvent having a dielectric constant of 46.6 (25°C) and a dipole moment of 3.9 D (25°C). It is a highly polar but aprotic solvent, which can mix well with any liquid. It is also called a super solvent and exhibits quite interesting properties.

Preparation of the Plates and Microbiological Assay

The antibacterial evaluation was done by Agar well diffusion method using Mueller Hinton Agar No.2 as the nutrient medium [15,16].



Reaction Scheme

The agar well diffusion method was preferred to be used in this study since it was found to be better than the disc diffusion method as suggested by Essawi and Srour [17]. The bacterial strains were activated by inoculating a loop full of test strain in 25ml of N-broth and the same was incubated for 24h in an incubator at 37° C. 0.2 ml of the activated strain was inoculated in Mueller Hinton Agar. Mueller Hinton Agar kept at 45°C was then poured in the Petri dishes and allowed to solidify. After solidification of the media, 0.85 cm ditch (well) was made in the plates using a sterile cork borer. Each well was filled with 0.1 ml of the test solution.

The plates were incubated for 24 h at 37°C. The mean value obtained for the three wells was used to calculate the zone of growth inhibition of each sample. The controls were maintained for each bacterial strain, where pure solvent (DMSO) was inoculated into the well. The inhibition zone formed by these compounds against the particular test bacterial strain determined the antibacterial activities of the synthetic compounds.

Results and Discussion

In all ten compounds were synthesized; The physical constants of all the synthesized compounds are shown in Table 1

Table I- Compound code, molecular formula, R, molecular weight, melting point, percentage yield, R_f value for the stated solvent system

Sr. No.	Code	R	M.F.	M. Wt. (g/mol)	R_f* Value	M.P. °C	Yield %
1	1	4-OCH_3-C_6H_4-	$C_{19}H_{15}ClFN_3O$	355	0.34	198	76
2	2	3-NO_2-C_6H_4-	$C_{18}H_{12}ClFN_4O_2$	371	0.46	165	72
3	3	2-OH-C_6H_4-	$C_{18}H_{13}ClFN_3O$	342	0.49	223	59
4	4	4-NH_2-C_6H_4-	$C_{18}H_{14}ClFN_4$	341	0.32	210	65
5	5	4-NO_2-C_6H_4-	$C_{18}H_{12}ClFN_4O_2$	371	0.41	202	69
6	6	3-NH_2-C_6H_4-	$C_{18}H_{14}ClFN_4$	341	0.43	189	64
7	7	4-CH_3-C_6H_4-	$C_{19}H_{15}ClFN_3$	340	0.33	165	66
8	8	4-OH-C_6H_4-	$C_{18}H_{13}ClFN_3O$	342	0.41	215	70
9	9	4-Cl-C_6H_4-	$C_{18}H_{12}Cl_2FN_3$	360	0.63	171	59
10	10	4-Br-C_6H_4-	$C_{18}H_{12}BrClFN_3$	405	0.43	188	66

**Acetone:Benzene: 2:8*

The IR, ^{1}H NMR and Mass spectral data of 2-chloro-6-fluoro-3-[3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-5-yl]quinoline is given below:

IR (KBr cm^{-1})

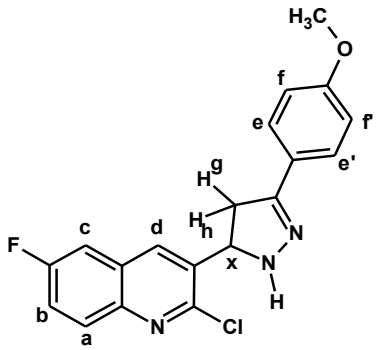
2926(alkane C-H str.(asym)), 2852(alkane C-H str.(sym.)), 3043 (aromatic C-H str.) 1516(aromatic C=C str.), 1031 (C-H i.p. def.), 827 (C-H o.o.p def.), 1607 (C=N str.), 1311 (C-N str.), 1216 (C-F str.), 739 (C-Cl str.), 1255 (N-H str.), 929 (N-N str.).

^{1}H NMR

2.91-2.97(2H, dd, Ar-Hg+Ar-Hh), 3.86(3H, s, -OCH_3), 3.78-3.85 (1H, m, Ar-Hx), 6.89-6.92(2H, dd, Ar-Hff'), 7.47-7.51 (2H, dd, Ar-Ha+Hb), 7.61-7.65(2H, m, Ar-Hee'), 7.98-8.01(1H, dd, Ar-Hc), 8.47 (1H, s, Ar-Hd).

Mass (m/z)

355, 320, 205, 188, 175, 160, 148, 133, 124, 77.



Antibacterial Activity

The microorganism used for detecting antimicrobial activity were chosen for the following reasons: the bacterium *S. aureus* was used due to its clinical relevance as a major cause of hospital acquired infections of surgical wounds and infections associated with indwelling medical devices. *S. aureus* rapidly develops resistance to many antimicrobial agents. *S. aureus* is responsible for skin infections[18]. *Bacillus cereus* is a human pathogen whose infections are amongst the most difficult to treat with conventional antibiotics[19]. *Staphylococcus epidermidis*, have emerged as major nosocomial pathogens associated with infections of implanted medical devices [20]. *Infections caused by S. epidermididis are often persistant and relapsing. M. luteus* is considered as an emerging nosocomial pathogen in immunocompromised patients. *E. coli* a gram negative usually motile is an extremely versatile opportunistic pathogen [21] causes septice mias and can infect the gall bladder, meningers, surgical wound, skin lesions and the lungs especially in debilitate and immunodeficient patients [22]. *Proteus mirabilis* which is a gram-negative motile rod, causes urinary track infections, wound infection also as often a secondary invader of ulcers, pressure sores, burns and damaged tissues and septicemia and occasionally meningitis and chest infections [21].

The antibacterial activity of 1-5 pyrazolines against Gram positive bacteria are shown in Fig. 1a while that of 6-10 pyrazolines against Gram positive bacteria are shown in Fig. 1b.

B. cereus was the most susceptible Gram positive bacteria while *M. luteus* was the most resistant Gram negative bacterial strain. All the 10 pyrazolines showed activity against *B. cereus* but to a varied level. Maximum activity was shown by compound No. 5, followed by compound No. 7 and 10. Minimum activity was shown by compound No. 8 and 9. When *S. aureus*, the most resistant Gram positive bacteria was considered, all the ten compounds showed almost similar activity. Only compound No. 5, 6, 7 and 10 showed antibacterial activity against *M. luteus*; compound No. 5 showed maximum activity. Compound No. 7 showed maximum activity against *S. epidermididis* while compound No. 6 and 9 showed the least activity.

When Gram negative bacterial strains are considered *E. coli* was the most resistant strain while *K. aerogenes* was the most susceptible strain. Compound No. 9 did not show activity against *P. mirabilis* while compound No. 8 did not show activity against *K. aerogenes*. Maximum activity was shown by compound No. 5 against *K. aerogenes* followed by No. 1 and No. 4; while all the compounds showed almost similar activity against *P. mirabilis*.

The obtained results showed different levels of activities against both Gram positive and Gram negative bacteria. Gram positive bacteria were found to be more susceptible than Gram negative bacteria. This could be due to the fact that the cell wall of Gram positive bacteria is less complex and lack the natural sieve effect against large molecules due to the small pores in their cell envelope[28]. Gram negative bacteria which are responsible for a large number of infectious diseases have a unique outer membrane that contains lipo polysaccharides which render them impermeable to certain antibacterial compounds [23].

Amongst the ten pyrazoline derivatives synthesized, only R is different in all the 10 compounds. It is observed that compound No. 5 which has nitro group at para position shows the best activity. This is followed by compound No. 7 and 10 which has para

methyl and para bromo groups respectively.

This differential activity of the compounds is because of the structural differences. The presence of nitro group enhances the activity. However, the methyl and bromide group attached at para position also increases the activity. The presence of nitro group at meta position decreases the activity which may be due to some steric hindrance. Other substituents are not very effective in inhibition.

A rapid and effective response to challenge pathogens is essential for the survival of all living organisms. The need for efficient agents increases with the expanding number of immunodeficient patients and with the emergence of bacterial and fungal pathogens resistant to current therapies [24].

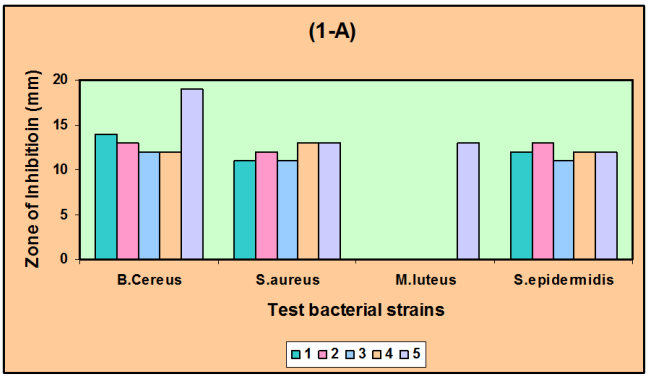


Fig. 1a- Antibacterial activity of pyrazolines against Gram+ve bacteria

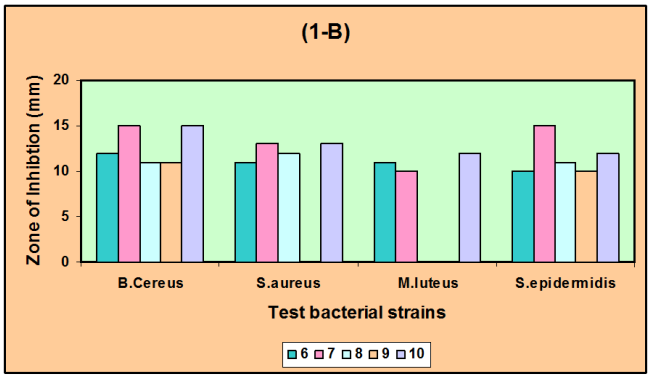


Fig. 1b- Antibacterial activity of pyrazolines against Gram+ve bacteria

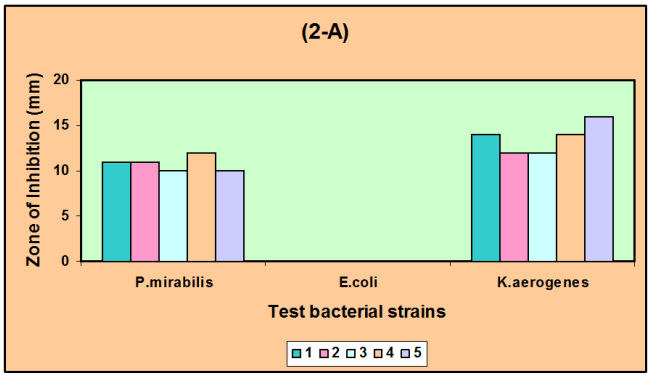


Fig.2a- Antibacterial activity of pyrazolines against Gram-ve bacteria

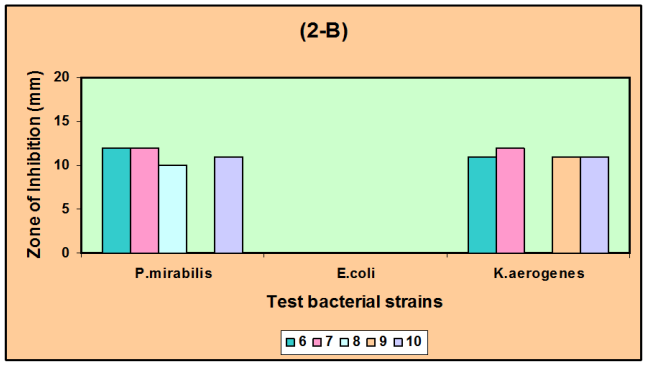


Fig.2b- Antibacterial activity of pyrazolines against Gram-ve bacteria

Conclusion

From the above results, it can be concluded that the pyrazoline derivatives which had nitro group showed best antibacterial activity provided the nitro group was at para position while the same nitro group at meta position decreased the activity. The next best was pyrazoline derivatives with methyl and bromo group at para position. These compounds showed better antibacterial activity towards Gram positive bacteria. *E. coli* was the most resistant bacteria and *B. cereus* was the most susceptible bacteria.

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