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Synthesis, characterization and antibacterial evaluation of novel 2-pyrazoline derivatives

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ABSTRACT

Several 3-(β-picolinoylaminoazo methyl-5-aromatic substituted)-1-thioamide-2-pyrazoline derivatives (4a-4e) were synthesized by reacting substituted 1-(β-picolinoylaminoazo)-3-benzylidene propan-2-one derivatives (3a-3e) with thiosemicarbazide in ethanol. The structures of the synthesized compounds were confirmed by FTIR, ^{1}H NMR, and EIMS spectral data. Their antimicrobial activities against E. coli (MTCC 442), P. Aeruginosa (MTCC 441), S. Aureus (MTCC 96), S. Pyrogenus (MTCC443) were investigated. A significant level of activity was observed.

Keywords: 2-Pyrazoline; Diazonium salt; Antibacterial agent.

INTRODUCTION

In the today's era most of antibacterials are chemically semi synthetic modifications of various natural compounds. These include, for example, the beta-lactam antibacterials, the cephalosporins, and the carbapenems. Compounds that are still isolated from living organisms are the aminoglycosides, whereas other antibacterials for example, the sulfonamides, the quinolones, and the oxazolidinones are produced solely by chemical synthesis. Most of the antibacterial compounds are classified on the basis of chemical/biosynthetic origin into natural, semisynthetic, and synthetic. Another classification system is based on biological activity; in this classification antibacterials are divided into two broad groups according to their biological effect on microorganisms: bactericidal agents kill bacteria, and bacteriostatic agents slow down or restrict bacterial growth.

Resistance against bacterial infections has resulted in the development of a wide variety of antibiotics. Bacteria are becoming antibiotic resistant after years of misuse and overuse of antibiotics, resulting in a potential global health crisis. There is already evidence that antibacterial resistance is associated with an increase in mortality. So to overcome this problem it is recommended to use new antibacterial agents with enhanced broad-spectrum potency. Therefore, recent efforts have been directed toward exploring novel antibacterial agents [1].

In order to overcome this rapid development of drug resistance, new agents should preferably consist of chemical characteristics that clearly differ from those of existing agents. In drug designing programs an essential component of the search for new leads is the synthesis of molecules, which are novel yet resemble known biologically active molecules by virtue of the presence of critical structural features. Certain small heterocyclic molecules act as highly functionalized scaffolds and are known pharmacophores of a number of biologically active and medicinally useful molecules [2-4].

Chemistry:

Pyrazolines are well known, and important nitrogen-containing 5-membered heterocyclic compounds and various methods have been worked out for their synthesis [5]. Numerous pyrazoline derivatives have been found to possess considerable biological activities. They have several prominent effects, such as antimicrobial [6,7], antimycobacterial [8], anti-inflammatory, analgesic [9,10], and antidepressant [11] activities. A large number of 2-pyrazolines using different synthetic methods for their preparation have been described in the chemistry literature. Most widely used procedure is based on the reaction of α,β-unsaturated aldehydes and ketones with hydrazines. However, a series of specially substituted representatives have been prepared rarely. For this reason, the aim of our present study was to synthesize systematically 3-(β-picolinoylaminoazo methyl-5-aromatic substituted)-1-thioamide-2-pyrazoline derivatives for the study of their antimicrobial activity.

Among the methods employed in synthesis of pyrazolines, condensation of a variety of substituted chalcones with hydrazine and its derivatives is commonly used. 2-Pyrazolines can be conveniently synthesized by the treatment of a ß-unsaturated carbonyl compounds with hydrazine reagents in basic and acidic media [12-14]. In this method, hydrazones are formed as intermediates, which can be subsequently cyclized to 2-Pyrazolines in the presence of thiosemicarbazide [15,16].

Biology:

The synthesized compounds were tested, at different concentration, for their in vitro antimicrobial activity against the gram-positive bacteria staphylococcus aureus, staphylococcus pyrogenus, the gram-negative bacteria escherichia coli, p. aeruginosa. The primary screen was carried out by agar disc-diffusion method[17] using nutrient agar medium. Ampicillin was used as control drug. The observed data on the antimicrobial activity of the compounds and control drug are given in Table 1.

MATERIALS AND METHODS

Melting points (m.p.) were determined in open capillary tube and are uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel G

(Merck). The necessary chemicals are of analytical grade and were purchased from local suppliers. Spectroscopic data were recorded with the following instruments; IR: Shimadzu 8400-FT-IR Spectrophotometer, ^{1}H-NMR: Varian Mercury YH-300 MHZ NMR spectrophotometer in $CDCl_3$ using TMS as an internal standard, and MS-FAB: Apiqstar-Pulsar Mass spectrometer.

*5.1.1 Synthesis of β-picolinoyl hydrazine **(1)**:*
Nicotinamide (40.9mM, 5g) was refluxed with (30mM, 1.5g) hydrazine hydrate in presence of 40ml of methanol at 100°-110°C for six hours. After the reaction mixture was cooled, filtered, and the separated product was purified by recrystallization from ethanol.

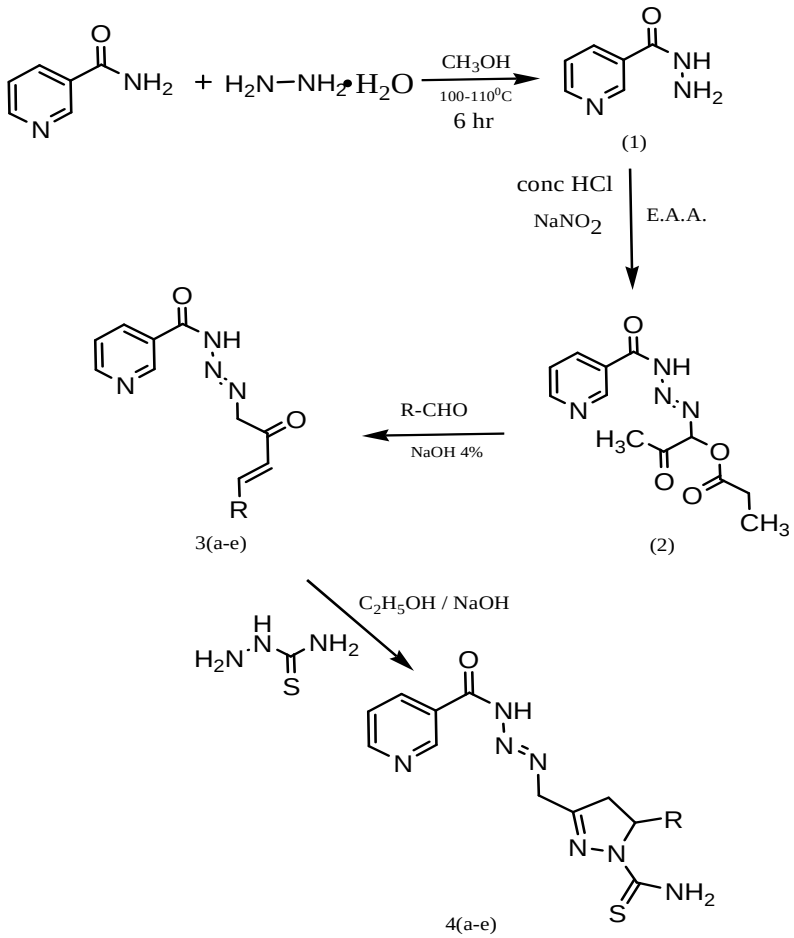
*5.1.2 Synthesis of 2-(β-picolinoylaminoazo)-ethyl aceto acetate **(2):***
β-picolinoyl hydrazine **(1)** (36.45mM, 5g) was dissolved in 5 ml of water and 5 ml of conc. HCl. After it was cooled to 0-5^{o}C in ice salt bath, cold aqueous solution of 6.9ml sodium nitrite in 8 ml of water was added drop wise to the above solution, diazonium Salt so formed was filtered into cold mixture of 13.5ml of ethyl aceto acetate and 4g of Sodium acetate in 25 ml of ethanol. The resulting solid was filtered and washed with water and it was recrystallized from ethanol.

*5.1.3 Synthesis of 1-(β-picolinoylaminoazo)-3-benzylidene propan-2-one derivatives **(3a-3e):***
2-(β-picolinoylaminoazo)-ethyl aceto acetate **(2)** (21.57mM, 6g) was added in different aldehydes (0.1M) in 20 ml of ethanol and add 3.2 ml of 4% sodium hydroxide solution. The mixture was stirred for 24hrs at room temperature. The contents were poured on crushed ice and neutralized with 10% HCl. The product was filtered, dried and recrystallized from ethanol.

*5.1.4 Synthesis of 3-(β-picolinoylaminoazo methyl-5-aromatic substituted)-1-thioamide-2-pyrazoline derivatives **(4a-4e):***
To a mixture of 1-(β-picolinoylaminoazo)-3-benzylidene propan-2-one **(3a-3e)** (0.01M) and sodium hydroxide (0.025M, 1g) in 50 ml of ethanol, thiosemicarbazide (0.12M, 10g) was added. The mixture was refluxed for 8hrs. The products were poured into crushed ice and the solid mass which separated out was filtered, dried and recrystallized from ethanol.

Analytical and spectral data (IR, ^{1}H-NMR, FAB-MS) confirmed the structures of the synthesized compounds.
3-{[(1E)-3-(pyridin-3-ylcarbonyl)triaz-1-en-1-yl]methyl}-4,5-dihydro-1H pyrazole-1-carbothio -amide (4a): Yield 82.48%; mp 172-175°C; IR (KBr) cm^{-1}: 1624.18(C=N stretching), 1785.27 (C=O stretching), 3000.69 (Ar-CH stretching), 3324.56 (N-H stretching), 1157.89 (C-N stretching), 1419.67 (C=S stretching); ^{1}H NMR (δppm) [300 MHz/$CDCl_3$]: 1.4 (2H- methylene), 2.7(H-methylene), 2.10 (2H amine), 8.11 (H- sec. amide) 8.84 (H-pyridine); MS: m/z 276(M^+), 78, 29, 165, 33, 91, 110.

3-[β-picolinoylaminoazomethyl-5-(2-hydroxy phenyl)]-1-thioamide-2-pyrazoline derivative (4b):
Yield 73.24%; mp 180-184°C; IR (KBr) cm^{-1} : 1624.18(C=N stretch), 1745.67 (C=O stretching), 3000.69 (Ar-CH stretching), 3330.56 (N-H stretching), 1157.89 (C-N stretching), 1424.67 (C=S stretching); ^{1}H NMR (δppm) [300 MHz/$CDCl_3$]: 1.1 (H- methyl), 1.4 (2H- methylene), 2.0 (2H-amine), 8.15 (H-sec amide), 8.57 (H- pyridine); MS: m/z 302 (M^+), 82, 78, 44, 42, 29, 16.



Scheme

4a: R= -H *4b: R= -CH_3* *4c: R= -C_3H_7* *4d: R= -$C_6H_5NO_2$* *4e: R= -C_6H_5*

3-[β-picolinoylaminoazomethyl-5-(phenyl)]-1-thioamide-2-pyrazoline derivative (4c): Yield 65.08%; mp 169-172°C; IR (KBr) cm^{-1} :. 1624.18(C=N stretching), 1720.67 (C=O stretching), 3000.73 (Ar-CH stretching), 3330.56 (N-H stretching), 1157.89 (C-N stretching), 1424.67 (C=S stretching); ^{1}H NMR (δppm) [300 MHz/$CDCl_3$]: 0.86 (3H methyl), 1.33 (2H methylene), 2.0 (2H amine), 8.38 (H- pyridine), 9.57 (H-pyridine); MS: m/z 335 (M^+), 198, 165, 111, 82, 78, 68.

3-[β-picolinoyl amino azomethyl-5-(4-methyl-5- amino-phenyl]-1-thioamide-2-pyrazoline derivative (4d): Yield 68.94%; mp 174-177°C; IR (KBr) cm^{-1} : 1419 (N=O stretching) 1624.18(C=N stretch), 1720.67 (C=O stretching), 3000.73 (Ar-CH stretching), 3330.56 (N-H stretching), 1157.89 (C-N stretching), 1424.67 (C=S stretching), ^{1}H NMR (δppm) [300MHz/$CDCl_3$]: 1.2 (2H methylene), 2.0 (2H amine), 7.21 (H- benzene), 9.0 (H- pyridine); MS: m/z 410(M^+), 122, 205, 198, 140, 162, 78, 68, 122, 29, 33.

5-phenyl-3-{[(1E)-3-(pyridin-3-ylcarbonyl)triaz-1-en-1-yl]methyl}-4,5-dihydro-1H-pyrazole-1-carbothioamide(4e): Yield 54.84%; mp 159-163 °C; IR (KBr) 1624.18(C=N stretching),

1780.42 (C=O stretching), 3000.73 (Ar-CH stretching), 3330.56 (N-H stretching), 1157.89 (C-N stretching), 1445.67 (C=S stretching); ^{1}H NMR (δppm) [300MHz/$CDCl_3$]: 1.20 (2H methylene), 1.98 (2H amine), 7.2 (H- benzene), 8.0 (H-sec amine), 8.9 (H pyridine); MS: m/z 362(M^+), 204, 163, 198, 140, 122, 111, 77, 68.

Biological assays:

Preliminary antimicrobial activities of 4a-4e compounds were tested by Agar cup plate diffusion method. Cups of (6 mm diameter) made in the agar plate and moistened with the test compound solution in DMF of specified concentration were carefully placed on the agar culture plates that had been previously inoculated separately with the microorganisms. The plates were incubated at 37° C and the diameter of the growth inhibition zones were measured after 24 h in case of bacteria.

RESULTS, DISCUSSION AND CONCLUSION

In this present work, a series of five new 2-pyrazoline derivatives were synthesized starting from β-picolinoyl hydrazine (1) according to the literature method.[18] Thus, we have obtained 2-(β-picolinoylaminoazo)-ethyl aceto acetate (2) through the treatment of (1) with sodium nitrite under diazotization reaction. The salt so formed was on reaction with different aldehydes forms 1-(β-picolinoylaminoazo)-3-benzylidene propan-2-one derivatives (3a-3e), which on further cyclization reaction with thiosemicarbazide forms 3-(β-picolinoylaminoazo methyl-5-aromatic substituted)-1-thioamide-2-pyrazoline derivatives (4a-4e). The IR spectra of the latter compounds showed, in each case, stretching band of C=O group in the region 1720-1780 cm^{-1}. Their ^{1}HNMR spectra revealed, in each case, the signal of sec. amine groups in the region 8.15-8.38 ppm, addition to the multiplet signal of CH–Pyridine in the region 8.57-9.57 ppm. Several authors have been reported the synthesis of 2-pyrazolines from the corresponding ketones.[19,20] In the same sense, the reaction of (3a-3e) with thiosemicarbazide in refluxing ethanol, in the presence of sodium hydroxide, afforded *3-(β-picolinoylaminoazo methyl-5-aromatic substituted)-1-thioamide-2-pyrazoline derivatives* (4a-4e) respectively. The structures of compounds 4a-4e were confirmed under the bases of their spectral data. In general, the inhibitory activity against the Gram-negative bacteria was higher than that of the Gram-positive bacteria. The *3-{[(1E)-3-(pyridin-3-ylcarbonyl)triaz-1-en-1-yl]methyl}-4,5-dihydro-1H pyrazole-1-carbothioamide (4a)* showed excellent activity against Gram-negative bacteria (inhibitory zone 23 mm), good activity against Gram-positive bacteria (inhibitory zone 21 mm). On the other hand, compounds 4b-4c showed better activities against S.Aureus and P.Aruginosa. Compound 4d showed a moderate activity against all the strains of bacteria. Compound 4e showed a good antibacterial activity against all the strains except a comparatively less activity against P. Aeruginosa.

Table 1: The in vitro antimicrobial activity of compounds

Sr.No	Derivative	Diameter of zone of inhibition (mm) for organisms			
		E.Coli MTCC 442	*P. Aeruginosa MTCC 441*	*S. Aureus MTCC 96*	*S .Pyrogenus MTCC443*
1	**4a**	23	20	23	21
2	**4b**	17	18	25	22
3	**4c**	12	23	16	19
4	**4d**	19	15	19	20
5	**4e**	20	11	20	20
6	**Ampicillin**	25	22	26	25

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