



SYNTHESIS, ANTI TUBERCULAR AND ANALGESIC ACTIVITY EVALUATION OF NEW 3-PYRAZOLINE DERIVATIVES

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ABSTRACT

An efficient synthetic method has been established for the synthesis of new 3-Pyrazoline derivative. 3- β -picolinoyl amino azo methyl-5-aromatic substituted-1-thioamide-3-pyrazoline was prepared by refluxing with thiosemicarbazide in presence of sodium hydroxide and ethanol. The structure of 3- β -picolinoyl amino azo methyl-5-aromatic substituted-1-thioamide-3-pyrazoline has been determined by IR and LCMSMS Spectroscopy. The synthesized compounds were evaluated for anti tubercular and analgesic activity.

Keywords: 3-pyrazolines, Anti tubercular activity, Analgesic and Anti-inflammatory activity.

INTRODUCTION

Drug therapy¹ for the treatment of TB has been greatly hampered by the development of MDR-TB and the lack of new classes of drug. In fact, no new drugs have been developed in the last 40 years. The only changes in the treatment of TB has been the strategy of using direct observed treatment, with an emphasis on patient centered care. Additionally, through the use of drug combinations, to 6 months, patient compliance continues to be serious problem, which in turn may be associated with the development of bacterial resistance.

The long term therapy required for the TB treatment which may result in drug resistance to first line agent and other severe complications such as haemolytic anaemia, agranulocytosis, liver damage and sometime crystalluria. In the last complication it may cause inflammation and pain. Thus present study emphasized on the synthesis of such compound which will have anti tubercular, analgesic and anti inflammatory activities coupled in one molecule and has less bacterial resistance.

Pyrazoline derivatives were found to have potential antipyretic² analgesic³, anti-inflammatory⁴, anti tubercular⁵ and antimicrobial⁶⁻¹² properties. They also found to exhibit a cytotoxic activity, inhibitory activity of platelet aggregation and herbicide activity¹³. 3-Pyrazolines have usually been prepared by starting from aldehydes or ketones, which have either actual or potential α,β -unsaturation¹⁴⁻¹⁷, 1,3-Dipolar cycloadditions between diazoalkanes and different types of molecules containing activated double bonds are also exploitable reactions¹⁸.

In this present synthetic work, we have synthesized new 3-pyrazoline derivatives from pyridine-3-carbonyl hydrazine as an important intermediate synthesized from nicotinamide as a starting material.

MATERIALS AND METHODS

The synthetic route of compounds is outlined in scheme 1.

Pyridine-3-carbonyl hydrazine¹⁹ (1) was prepared by refluxing Nicotinamide with Hydrazine hydrate in methanol. 2- β -picolinoyl amino azo ethyl aceto acetate²⁰ (2) was synthesized by refluxing pyridine 3-carbonyl hydrazine (1) with ethyl aceto acetate.

The 1- β -picolinoyl amino azo methyl benzylidene substituted propane 2-one derivatives²⁰ (3) were prepared by refluxing compound (2) with benzaldehyde. The condensation of 1- β -picolinoyl amino azo methyl benzylidene substituted propane 2-one (3) with thiosemicarbazide result in the formation of 3- β -picolinoyl amino azo methyl-5-aromatic substituted-1-thioamide-3-pyrazoline derivatives⁶ (4a-e).

Experimental data of some compounds are shown in the Table 1. Spectral data (¹H NMR and EIMS) confirmed the structure of new synthesized compounds.

Melting points were determined by open capillary method and are uncorrected. IR, ¹H NMR and EIMS spectra of synthesized compounds were recorded on Shimadzu 8400-S-FT-IR, VARIAN MERCURY YZ-300MHZ NMR recorded at University of Pune, India and API QSTAR-PULSAR recorded at NCL, Pune.

SYNTHESIS OF PYRIDINE-3-CARBONYL HYDRAZINE (1)

Nicotinamide (0.1M, 10.6g) was refluxed with (0.1M, 3.2g) Hydrazine hydrate in presence of methanol (1M, 40ml) at 100°-110°C for four hours. After the reaction mixture was cooled, filtered, and the separated product was purified by recrystallized from ethanol.

Synthesis of 2- β -picolinoyl amino azo ethyl aceto acetate from pyridine 3-carbonyl hydrazine (2)

Pyridine 3-carbonyl hydrazine (0.1M, 13.6g) was dissolved in water (5ml), Hcl (5ml). After it was cooled to 0-5°C in ice salt bath, cold aq. solution of sodium nitrite (0.01M, 4.3ml) in water (8ml) was added drop wise to the above solution, diazonium Salt so formed was filtered into cold mixture of ethyl aceto acetate (0.01M, 13.5ml) and Sodium acetate (4g) in ethanol (25ml).

Synthesis of 1- β -picolinoyl amino azo methyl benzylidene substituted propane 2-one derivatives (3)

2- β -picolinoyl amino azo ethyl acetoacetate (0.1M, 27.8g) was added in benzaldehyde in ethanol (20ml) and add 4% sodium hydroxide Solution. The mixture was stirred for 24hrs at room temp. The contents were poured on crushed ice and neutralized with 10% Hcl. The product was filtered, dried and crystallized from ethanol.

Synthesis of 3- β -picolinoyl amino azo methyl-5-aromatic substituted-1-thioamide-3-pyrazoline derivatives (4a-e)

To a mixture of 1- β -picolinoyl amino azo methyl benzylidene substituted Propane 2-one (0.01 mol) and sodium hydroxide (1g, 0.025 mol) in ethanol (50mL), thiosemicarbazide (0.012 mol) was added. The mixture was refluxed for 8 h. The products were poured into crushed ice and the solid mass which separated out was filtered, dried and crystallized from ethanol.

4a: IR (KBr) cm^{-1} : 1619.91, 1533.13(Ar.stretch); 1644(C=O str.); 3262.97(-NH stretch); 1619.91(C=N str.); 1001.84(C=S stretch); 3367.1(-NH str.). ¹H NMR (δ ppm) [300 MHz/DMSO]: 6.91-9.11 (8H,m,ar,py.), 3.89 (2H,s,Pyrazoline), 2.88-3.54 (2H,dd,pyr.), 3.74 (3H,s,-OCH₃), 8.50 (3H,s,pyr.), 5.62-5.64 (1H,d,pyr.). MS (EIMS): m/z 435.43(M⁺), 109.13, 133.12, 155.01, 169.02, 225.20, 279.10, 323.13, 338.11.

4b: IR (KBr) cm^{-1} : 3178.45, 1619.91, 1533.13(-CH-stretch, Ar); 1619.91(C=N str.); 3263.93(-NH stretch); 1747.19(C=O str.); 999.91(C=S stretch); 3370.96(OH- str.). ^1H NMR (δ ppm) [300 MHz/DMSO]: 6.83-8.71 (8H,m,ar,py.), 3.89 (2H,s,Pyrazoline), 2.90-3.56 (2H,m,pyr.), 5.87 (1H,d,pyr.), 8.03 (4H,s,-NH). MS (EIMS): m/z 421.21 (M^+), 122.32, 133.07, 155.01, 169.02, 211.08, 263.07, 304.25, 323.13, 387.13.

4c: IR (KBr) cm^{-1} : 1635.02(-CH-stretch, Ar); 1321(>N- str.); 1635.02(-C=N-stretch); 1749.62, 1635.02(C=O str.); 1039.41(C=S stretch); 1036.22(NH_2 - str.). ^1H NMR (δ ppm) [300 MHz/DMSO]: 7.15-9.10(8H,m,ar,py.), 3.89 (2H,s,Pyrazoline), 2.85-3.58 (2H,m,pyr.), 5.60-5.66 (1H,t,pyr.), 2.85-3.58 (2H,m,-NH). MS (EIMS): m/z 390.13 (M^+), 119.10, 133.11, 155.01, 258.011, 274.08, 291.09, 304.34, 323.13.

4d: IR (KBr) cm^{-1} : 1509.67, 1491.24(-CH-stretch, Ar); 1314.28(>N-str.); 1635.02(-C=N-stretch); 1749.62, 1826.72, 1635.02(C=O str.); 997.23(C=S stretch); 1067.28(C-N-str.). ^1H NMR (δ ppm) [300MHz/DMSO]: 6.88-9.11(8H,m,ar,py.), 3.89 (2H,s,Pyrazoline), 2.84-3.57 (2H,m,pyr.), 5.69 (1H,d,pyr.), 7.65 (5H,s,-NH). MS (EIMS): m/z 413.43 (M^+), 119.10, 133.12, 195.053, 241.21, 291.023, 342.11, 405.99, 433.023.

4e: IR (KBr) cm^{-1} : 3178.11, 1619.91, 1533.13, 1485.88(-CH-stretch, Ar); 3262.97(NH- str.); 1619.22(-C=N-stretch); 3371.92, 3262.97(OH-str.); 997.98(C=S stretch); 1289.18(C-O-str.). ^1H NMR (δ ppm) [300MHz/DMSO]: 6.48-9.11(8H,m,ar,py.), 3.89 (2H,s,Pyrazoline), 2.68-3.51 (2H,m,pyr.), 5.60 (1H,d,pyr.), 7.91 (4H,s,-NH). MS (EIMS): m/z 437.21 (M^+), 119.10, 133.12, 155.01, 279.10, 282.28, 304.34, 323.13, 409.04, 437.21.

EXPERIMENTAL

Anti tubercular activity

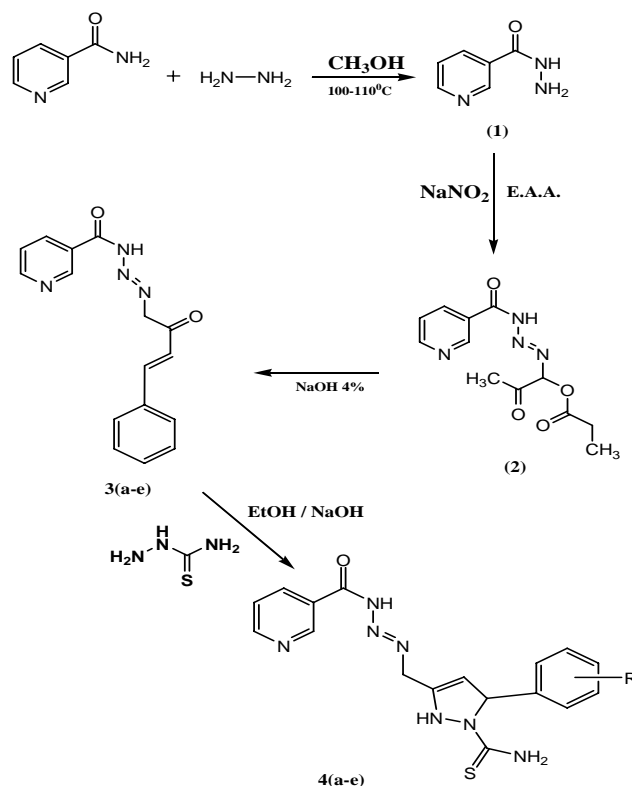
Anti tubercular activity of compounds were tested against *Mycobacterium tuberculosis* by using REMA plate method²¹. Isoniazid was used as control drug. The observed data on anti tubercular activity of the compounds are given in Table 2.

Analgesic activity

Acetic acid induced writhing in mice method²² was used to determine analgesic activity in vivo. Analgesic activity was determined by calculating total number of writhings, following intraperitoneal (I.P) administration of 0.6% (0.1ml/10 g) acetic acid in mice. Albino mice of either sex (25-30 g) were used. Synthesized compounds (4a- 4e) were administered intraperitoneally (0.5mL) as a suspension in sterile 0.9% DMSO solution as vehicle. Diclofenac sodium (10 mg/kg) was used as the standard drug under same conditions. Acetic acid solution was administered intraperitoneally 30 min after administration of the compounds. 10 min after intraperitoneal injection of the acetic acid solution, the number of writhings per animal was recorded for 20 min. Control animals received an equal volume of vehicle.

Results of Percentage Analgesic activity of compounds was calculated using following formula and the results are shown in Table-3.

$$\% \text{ Analgesic activity} = \left[\frac{\text{No. of writhings for control} - \text{No. of writhings for test compound}}{\text{No. of writhings for control}} \right] \times 100$$



R = 2-NO₂ (4a), 4-NO₂ (4b), 4-Cl (4c), 3-OCH₃ (4d)

4-OCH₃ (4e)

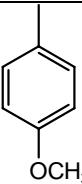
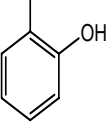
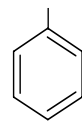
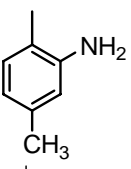
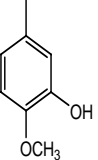
Scheme 1.

RESULTS AND DISCUSSION

The series of compounds obtained (4a-e) were evaluated for anti tubercular and analgesic activity against REMA plate method and acetic acid induced writhing (modified Koster's test)^{9,10}. Isoniazid, Diclofenac were used as the standards in above cited tests respectively. The synthesized compounds (4a-e) exhibited good anti

tubercular activity at various MIC levels. Among them, compounds 4a, 4c, and 4e exhibited good antitubercular activity against *Mycobacterium tuberculosis* but less as compared with the standard. The compounds 4b, 4d and 4e shows good analgesic activity and compounds 4b and 4c shows moderate activity. The results summarized in Table 2, Table 3.

Table 1: Physicochemical data of compound (4a-e)

Compound	R	Molecular formula	Molecular weight	M.P. (°C)	Rf value	Yield (%)
4a		C ₁₈ H ₁₉ N ₇ O ₂ S	397.9	170-172°C	0.80	82.48
4b		C ₁₇ H ₁₇ N ₇ O ₂ S	381.9	178-180°C	0.90	73.24
4c		C ₁₇ H ₁₇ N ₇ OS	367.9	167-169°C	0.76	65.08
4d		C ₁₈ H ₁₈ N ₈ O ₂ S	409	172-174°C	0.62	68.94
4e		C ₁₈ H ₁₉ N ₇ O ₃ S	413.5	157-159°C	0.43	54.84

• Recrystallisation solvent - Ethanol.

Table 2: Anti tubercular activity of 4a to 4e

Comd.	Conc. of Test sample/ minimum inhibitory concentration (MIC) colour change							
	1.25 mg/mL	2.5 mg/mL	3.75 mg/mL	5.0 mg/mL	6.25 mg/mL	7.5 mg/mL	8.75 mg/mL	10.0 mg/mL
4a	-----	Blue Colour to Pink	-----	-----	-----	-----	-----	-----
4b	-----	-----	Blue Colour to Pink	-----	-----	-----	-----	-----
4c	Blue Colour to Pink	-----	-----	-----	-----	-----	-----	-----
4d	-----	-----	-----	-----	Blue Colour to Pink	-----	-----	-----
4e	-----	Blue Colour to Pink	-----	-----	-----	-----	-----	-----

Table 3: Acetic acid induced writhing response in mice for (4a-4e)

Sr. No	Derivative	Dosage	Number of writhings in 20 minutes mean ± S.E.M)	% Analgesic* activity
1	Control	Vehicle	66.5 ± 5.98	0
2	4a	20 mg/kg	22.33 ± 4.30	67.32***
3	4b	20 mg/kg	8.5 ± 1.88	88.21***
4	4c	20 mg/kg	22.66 ± 7.77	59.42***
5	4d	20 mg/kg	10.5 ± 2.57	74.21***
6	4e	20 mg/kg	10.16 ± 1.01	89.72***
7	Diclofenac	20 mg/kg	4.66 ± 2.06	91.99****

N=6; student t test; * P≤0.05; ** P≤0.01; ***P≤0.001 when compared with control.

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