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Synthesis, antitubercular, anticonvulsant and antiinflammatory activities of some novel 2-pyrazoline derivatives

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ABSTRACT

Inflammation of lungs is often related with the tuberculosis and convulsions are associated with the long term therapy of first line anti tubercular agents. Here in this present study an attempt has been made to synthesize the derivatives possesses all three activities. An efficient synthetic method has been established for the synthesis of new 2-Pyrazoline derivative. The synthesized compounds were evaluated for anti tubercular, anticonvulsant and anti-inflammatory activity. The observed increase in activities are attributed to the presence of 2-CH_3, 4-NH_2 in phenyl ring at 5-position of pyrazoline ring of newly synthesized compounds.

Key words: Synthesis, pyrazoline derivatives, tuberculosis.

INTRODUCTION

Drug therapy[1] for the treatment of tuberculosis 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 convulsions and inflammation. Thus present study emphasized on the synthesis of such compound which will have anti tubercular, anticonvulsant and anti inflammatory activities coupled in one molecule and has less bacterial resistance. Pyrazoline derivatives were found to have potential anticonvulsant[2] analgesic[3], anti-inflammatory[4], anti tubercular[5] and antimicrobial[6] properties. In view of above, an attempt has been undertaken to synthesize new 3-(β-picolinoylaminoazomethyl-5-aromaticsubstituted)-1-thioamide-2-pyrazoline derivatives possessing anti-tubercular, anticonvulsant and anti-inflammatory activities.

MATERIAL AND METHODS

All the chemicals used in this synthesis were of laboratory grade. Sodium hydroxide, nicotinamide, hydrazine hydrate, benzaldehyde, p-methyl amino benzaldehyde, anisaldehyde, salicylaldehyde, carrageenan (all from Merck, Germany).

The melting points were determined in open capillary method and are uncorrected. The

synthesized compounds were purified by recrystallization by using methanol as solvent. The purity of the compounds was confirmed by TLC methods. The IR spectra of synthesized compounds were recorded on Shimadzu 8400-S FT-IR Spectrophotometer using potassium bromide.^{1}H NMR spectra were recorded on VARIAN MERCURY YZ-300MHZ NMR spectrophotometer**.** Using DMSO as solvent and chemical shifts are given in parts per million, downfield from tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on APIQSTAR-PULSAR using methanol as solvent.

EXPERIMENTAL

Synthesis of β-picolinoyl hydrazine (1)

Nicotinamide (0.1M, 12.2g) was refluxed with (0.1M, 3.2g) 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. Melting point and % yield and molecular formula are given in Table-1.

Synthesis of 2-(β-picolinoylaminoazo)-ethyl aceto acetate (2)

β-picolinoyl hydrazine **(1)** (0.1M, 13.6g) was dissolved in 5 ml of water and 5 ml of Hcl. After it was cooled to 0-5°C in ice salt bath, cold aq. 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. Melting point and % yield and molecular formula are given in Table-1.

Synthesis of 1-(β-picolinoylaminoazo)-3-benzylidene propan-2-one derivatives (3a-3e)

2-(β-picolinoylaminoazo)-ethyl aceto acetate (2) (0.1M, 27.8g) was added in different aromatic aldehydes in 20 ml of ethanol and add 4% sodium hydroxide Solution. The mixture was stirred for 24hrs at room tempt. The contents were poured on crushed ice and neutralized with 10% Hcl. The product was filtered, dried and recrystallized from ethanol. Their melting points and % yields and molecular formula are given in Table-1.

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, 13.4g) 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. Their melting points and % yields and molecular formula are given in Table 1.

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.). ^{1}H NMR (δppm) [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,-OCH_3), 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.). ^{1}H NMR (δppm) [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.). ^{1}H NMR (δppm) [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.);

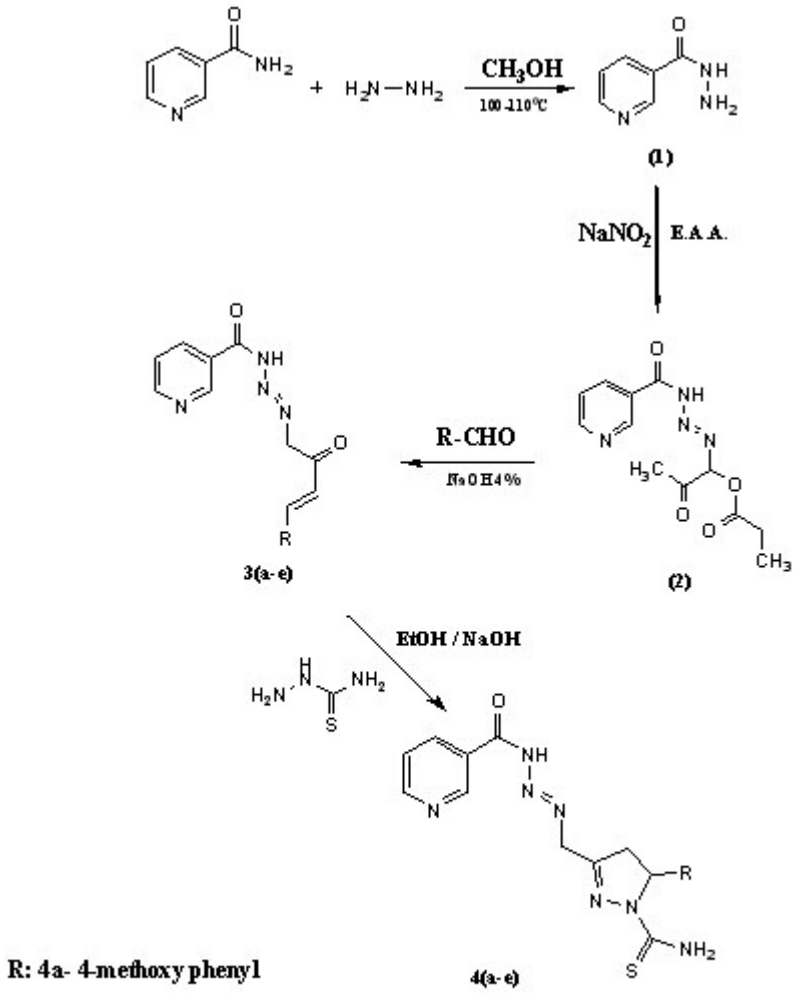
997.23(C=S stretch); 1067.28(C-N-str.). ^{1}H NMR (δppm) [300MHz/DMSO]: 6.88-9.11(8H,m,ar,py.), 3.89 (2H,s,Pyrazoline), 2.84-3.57 (2H,m,pyr.), 5.69 (1H,d,pyr.), 7.65 (5H,s,-NH). MS (EIMS): m/z 413.43 (M^+), 119.10, 133.12, 195.053, 241.21, 291.023, 342.11, 405.99, 433.023.

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.). ^{1}H NMR (δppm) [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.

Anti tubercular activity

Anti tubercular activity of compounds were tested against *Mycobacterium tuberculosis* $H37R_V$ using REMA plate method[7]. Isoniazid was used as control drug. The observed data on anti tubercular activity of the compounds are given in Table 2.



Scheme 1.

Animals

Wistar albino mice (18-22 g) and Swiss albino rats (120-150 g) of either sex were selected. The principles of Laboratory Animal Care (NIH, 1985) were followed and instructions given by our institutional animal ethical committee were maintained throughout the experiment. The pharmacological activities were performed under the proposal number of CPCSEA, NCP/IAEC/PG-43/ 2009.

Anticonvulsant activity

Anticonvulsant activity[2] was determined by Isoniazid induced convulsion test. Six mice in each group of either sex with a weight of 18 to 22 gm are taken. Group I control animals received 30% aqueous PEG 400 only, group II received standard (diazepam 10 mg/kg) i.p. Group III-VII are treated with the test compound (4a-4e) by oral administration. The synthesized compounds (4a-4e) were suspended in 30% aqueous solution of PEG 400 and administered orally in a standard volume of 0.5 ml/20 g body weight at 30 mg kg^{-1} doses. 30 min after i.p. or 60 min after p.o. treatment the animals are injected with a subcutaneous dose of 300 mg/kg isoniazid (isonicotinic acid hydrazide).The occurrence of clonic seizures, tonic seizures and death is recorded. Anticonvulsant activity was expressed as percentage of tonus and clonus mortality. The results are presented in Table-3.

Anti inflammatory activity

The anti-inflammatory activity[8] was determined by carrageenan-induced paw edema in rats. For this experiment, the male rats (120-150g) were divided into four groups (n= 6). The first group received 0.5% CMC (10ml/ kg p.o.), while the second group received Indomethacin (100 mg/kg p.o). The third and the fourth groups were treated with the synthesized compounds (100 mg/ kg, p.o.) respectively. Acute inflammation was produced by the sub plantar administration of 0.1 ml of 1% Carrageenan (in 1% CMC w/v) in the right hind paw of the rats. The paw thickness was measured at 0 min, 30 min, 60 min, 120 min and 240 min after Carrageenan injection by using verniar calipers. The animals were pretreated with the drug 1 hour before the administration of Carrageenan. The results are presented in Table 4.

Statistical analysis

Data were analyzed by one-way ANOVA followed by Dunnett's *t*-test using computerized Graph Pad Instat version 3.05 (Graph Pad software, U.S.A.).

RESULTS AND DISCUSSION

The series of compounds obtained (4a-e) were evaluated for anti tubercular, anti-convulant and anti-inflammatory activity against REMA plate method, Isoniazid induced convulsion test and

Table 1: Characterization data of compounds 1, 2, 3a-3e and 4a-4e

Comp.	Molecular formula	Molecular weight	% Yield (w/w)	Melting point(°c)
1	$C_6H_7N_3O.$	137.14	72.14	218-220
2	$C_{12}H_{14}N_4O_4$	278.26	62.82	181-183
3a	$C_{17}H_{17}N_5O_2$	323.35	58.82	105-108
3b	$C_{16}H_{15}N_5O_2$	309.32	69.85	125-128
3c	$C_{16}H_{15}N_5O$	293.32	73.52	117-119
3d	$C_{17}H_{16}N_6O_2$	336.35	63.23	108-110
3e	$C_{17}H_{17}N_5O_3$	339.35	74.26	131-135
4a	$C_{18}H_{19}N_7O_2S$	397.9	82.48	170-172
4b	$C_{17}H_{17}N_7O_2S$	381.9	73.24	178-180
4c	$C_{17}H_{17}N_7OS$	367.9	65.08	167-169
4d	$C_{18}H_{18}N_8O_2S$	409	68.94	172-174
4e	$C_{18}H_{19}N_7O_3S$	413.5	54.84	157-159

Carrageenan—induced paw edema respectively. Isoniazid, Diazepam and Indomethacin were used as the standards in above cited tests respectively.

Anti-tubercular activity

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 results are shown in Table 2.

Anti-convulsant activity

All the compounds i.e. 4a to 4e showed a significant decrease in % of latency of clonus and % of tonus and clonus mortality. The compounds 4c, 4d showed excellent anticonvulsant activity and compound 4a and 4e showed moderate activity. The results are shown in Table 3.

Table 2: Anti tubercular activity of synthesized compounds against *Mycobacterium tuberculosis* $H37R_v$

Compound	MIC Concentration (µg/ml)
4a	2.5
4b	3.75
4c	1.25
4d	6.25
4e	2.5

Table 3: Anticonvulsant activity of compounds (4a-4e)

Groups	Latency of clonus (min.)	% of clonus	% of tonus and clonus mortlity
Control	3.20 ± 0.1797	100	100
Standard	8.57 ± 0.2144**	100	0
4a	4.03 ± 0.1806*	100	66.67
4b	4.52 ± 0.1366	100	83.34
4c	7.53 ± 0.1438**	100	16.57
4d	8.15 ± 0.0551**	100	16.67
4e	6.40 ± 17.84*	100	50.00

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

Table 4: Carrageen induced inflammation in rat model for (4a-4e)

S. No	Derivative	Dosage	% Decrease in inflammation (mean)
1.	Control	Vehicle	-
2.	Indomethacin	2 mg/kg	66.12***
3.	4a	20 mg/kg	35.1**
4.	4b	20 mg/kg	52**
5.	4c	20 mg/kg	56.32*
6.	4d	20 mg/kg	39*
7.	4e	20 mg/kg	34.5**

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

Anti-inflammatory activity

All the compounds i.e. 4a to 4e showed a significant decrease in inflammation. Moreover, the compounds 4b and 4c were found to also bring about a decrease in inflammation comparable to that of standard (Indomethacin). The results obtained are showed in the Table 4.

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