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Synthesis, Characterization & Evaluation of Some Novel Pyrazoline Derivatives as Anti-Tuberculosis Agents

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Abstract : In the present investigation, a series of 4-(4-(dimethylamino) benzyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one analogues of pyrazole were synthesized by cyclization of mannich base with hydrazine hydrate. The nitrogen functionality of 4-(4-(dimethylamino) benzyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one was alkylated with different halogenated alkylated substituted chain in the presence of base. The chemical structure of compounds were proved by IR, MASS, ^{1}HNMR spectroscopy data and elemental analysis. The antitubercular activity of these compounds were evaluated by microdilution method against *M. Tuberculosis* $H_{37}Rv$ micobactrium strain of M. Tuberculosis compared with standard INH, RIF & ETM.
Keywords: Antituberculosis, Isoniazid, Rifampicin, Pyrazoline.

Introduction

Mycobacterium tuberculosis (MTB), the pathogenic agent of tuberculosis (TB), is responsible for the death of 2–3 million people annually and for a global economic toll of w$12 billion each year[1]. The rise of multidrug resistance (MDR) in MTB has complicated and prolonged the treatment[2]. No new drugs have been developed specifically against mycobacteria since the 1960s and only within the last few years have some promising drug candidates emerged[3,4]. Thus, there is an urgent need to develop new therapeutics for tuberculosis, to reduce the duration of treatment and to provide a more effective therapy for MDR TB and for latent tuberculosis infection[5]. In most parts of the world, we are limited to combinations of five drugs to treat

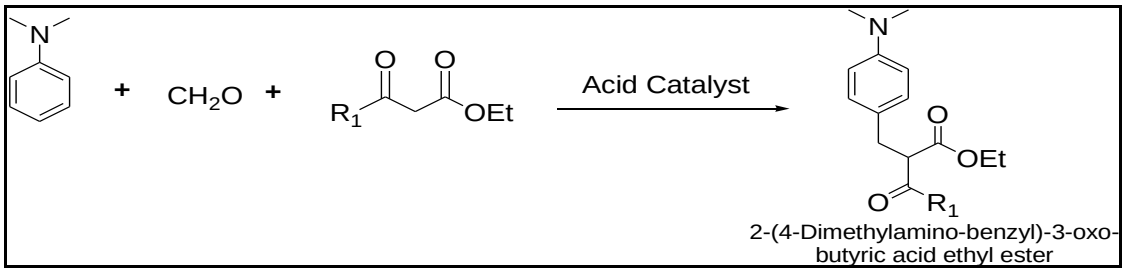
TB effectively, namely rifampicin, isoniazid (INH), ethambutol, streptomycin and pyrazinamide. Problems in the chemotherapy of tuberculosis arise when patients develop bacterial resistance to any of these first-line drugs and because second-line drugs, such as ethionamide, aminosalicylic acid, cycloserine, amikacin, kanamycin and capreomycin are too toxic and cannot be employed simultaneously[6]. Resistance of Mycobacterium tuberculosis (MTB) strains to antimycobacterial agents is an increasing problem worldwide[7-9]. Previously reported that certain pyrazoline are potent inhibitor of mycobacterium tuberculosis (M.tb) in vitro[10-13]. These compound display several properties which make them highly interesting as potential drug against tuberculosis: highly selective towards Mtb compared to other microorganism, activity against several drug resistant strains of Mtb, low toxicity toward mammalian cells, ability to affect Mtb inside macrophages. So to obtain more selective compounds with high efficiency, we synthesized some pyrazoline derivatives which can serve as the novel targets for treatment against M.tb.

Chemistry

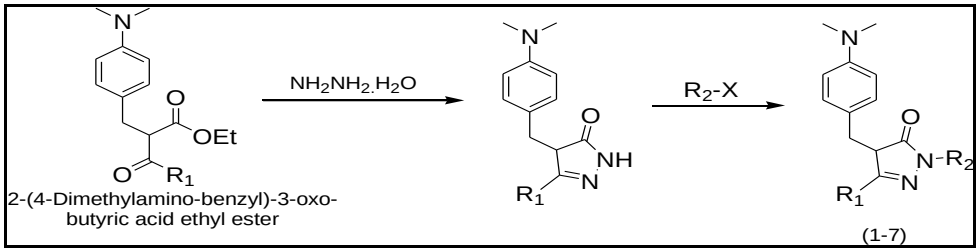
A series of 4-(4-(dimethylamino) benzyl)-3-methyl-1H-pyrazol-5(4H)-one (1-7) derivatives were prepared through mannich base and hydrazine hydrate. The nitrogen functionality of pyrazoline was alkylated with different halogenated alkylated substituted chain, to synthesize of novel pyrazoline (1-7) in 70-93% yield.

The purity of compounds was checked by TLC and elemental analysis. The structure of newly synthesized compounds were confirmed by IR, MASS, ^{1}HNMR. Final compounds in general, in the infrared spectra (IR), revealed C=C, C-H, C=O, C-O, N-H, and C=N peaks at 1516, 3020, 1710, 1275, 3183 and 3183 cm^{-1}, respectively. In the nuclear magnetic resonance spectra (^{1}HNMR) the signals of the respective protons of the prepared titled compounds were verified on the basis of their chemical shifts, multiplicityes and coupling constant.

Scheme 1



Scheme 2



Where, R_1 = Alkyl group R_2 = Alkyl group, Aryl group

Experimental Protocol

All the reagents were procured from Sigma-Aldrich Chemical Co, Lancaster and were used directly without any further purification. Melting ranges were determined in open capillaries on COMPLAB melting point apparatus and are otherwise uncorrected. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm precoated plate of silica gel $60F_{254}$, E. Merck, Darmstadt, Germany and the detection was done by iodine and UV radiation. Compounds were purified by column chromatography performed with silica gel (Merck, 10-200 mesh). ^{1}HNMR spectra (ppm, δ) were recorded on Brucker ADVANCE DPX 300 MHz/200MHz spectrometer with TMS as the internal standard. Mass spectra were recorded by using JEOL-SX-102/ DA-6000 instrument using electrospray ionisation ESI method. Infrared spectra (ν_{max} in cm^{-1}) were recorded on Beckman Aculab-10, Perkin Elmer 881, FTIR 8210 PC and Shimadzu spectrophotometer either in KBr or in Neat. Elemental analyses were performed on Perkin Elmer Auto System XL Analyzer.

General procedure

(a) Synthesis of 2-(4-dimethylamino-benzyl)-3-oxo butyric acid ethyl ester (I)

To a mixture of N, N-dimethyl-aniline (0.008mol, 1.05 ml.), ethylacetoacetate (0.012 mol, 1.62 ml) and glacial acetic acid (3ml.) were added in a 100 ml. RBF and reaction mixture was stirred on magnetic stirrer at room temperature. After 30 min., formaldehyde (0.024 mol, 0.663 ml.) was added drop-wise and progress of reaction was monitored by TLC in solvent system (20% e14thyl acetate and n-hexane). The reaction was completed in 7 hrs and extracted with ethyl acetate and water. The organic phase was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. The compound was purified by silica gel (100-200 mesh size) column chromatography. Solubility – ethyl acetate, dichloromethane. ^{1}H-NMR($CDCl_3$, 300MHZ)- δ 7.0744(d, J=8.55Hz, 2H); 6.6845(d, J=8.61Hz, 2H); 4.15-4.20(m, 2H, CH2); 3.758(t, 1H); 3.100(d,2H, J=7.62, CH2 Ar); 2.923(s, 6H, CH); 2.188(s, 3H, CH); 1.21-1.30(M, 3H). Mass (m/e)- 264(m+1).

IR (Neat)- C=O (1725-1705) 1713.4, C-H (3000-2850), C=C (1600-1475) 1578, C-H (3150-3050) 3020, C-N (1350-1000) 1216, C-O (1300-1000) 1045. RF value – 0.7.

b) Synthesis of 4-(4-(dimethylamino) benzyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one (II)

To a mixture of (**I)** (0.0049 mol, 1.30 gm.) and hydrazine hydrate (0.014 mol, 0.721 ml.) in 100 ml. RBF and 2 ml. of ethanol were added and reaction mixture was refluxed on water bath at 70-80 ^{0}C. The progress of reaction was monitored by TLC (4% methanol-chloroform). Reaction was completed in 3 hrs. Reaction mixture was cooled, filtered, and washed with methanol and water. The compound was recrystallised with methanol., ^{1}HNMR($CDCl_3$, 300MHz)- δ 6.978(d, J=8.55Hz, 2H); 6.627(d, J=8.64Hz, 2H); 3.41(d, J=14.07Hz, 2H); 2.808(s, 6H, CH); 2.509(t, 2H, CH2); 1.979(s, 3H); Mass (m/e)- Mol. Wt. – 230, (m+1) –231; IR (KBr)- C=O (1725-1705) 1710, C-H (3000-2850) 2921, C=C (1600-1475) 1516, C-H (3150-3050) 3020, C=N (1690-1600) 1612, C-O (1300-1000) 1275, 1209, N-H (3500-3100) 3183; RF value – 0.5.

1. General procedure synthesis of 4-(4-(dimethylamino) benzyl)-3-methyl-1-(2- (piperidin-1-yl) ethyl)-1*H*-pyrazol-5(4*H*)-one

To a mixture of **(II)** (0.001mol, 0.231 gm.) and 2-chloro ethyl piperidine hydro chloride (0.0012 mol, 0.220 gm.) in anhydrous dimethyl formamide and dry K_2CO_3 (0.01 mol, 1.38 gm.) were added in 100 ml RBF and reaction mixture was refluxed on water bath at 80-90^{0}C[5]. The reaction was monitored by TLC (10% methanol-chloroform). The reaction was completed in 10 hrs and extracted with ethyl acetate and water. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The compound was purified by silica gel (100-200 mesh size) column chromatography. ^{1}HNMR($CDCl_3$, 300MHz)- δ 6.978(d, J=8.55Hz, 2H); 6.627(d, J=8.64Hz, 2H); 3.41(d, J=14.07Hz, 2H); 2.808(s, 6H, CH); 2.509(t, 2H, CH_2); 1.979(s, 3H). Mass (m/e)- Mol. Wt. – 230, (m+1) –231. IR (KBr)- C=O (1725-1705) 1710, C-H (3000-2850) 2921, C=C (1600-1475) 1516, C-H (3150-3050) 3020, C=N (1690-1600) 1612, C-O (1300-1000) 1275, 1209, N-H (3500-3100) 3183. RF value – 0.5

2.Synthesis of 4-(4-(dimethylamino) benzyl)-1-(3-(dimethylamino) propyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one

^{1}HNMR ($CDCl_3$, 300 MHz)- δ 7.085-6.939 (m, 2H); 6.689-6.574 (m, 2H); 4.224(t, 2H); 2.900(s, 6H); 2.807(s, 3H); 2.478(t, 2H); 2.281(s, 6H); 2.190(d, J=1.2Hz, 1H); 1.968(t, 2H); 0.895(t, 2H). Mass (m/e)- Mol. Wt. - 316, (m+1) – 317. IR (Neat) - C=O (1725-1705) 1724, C-H (3000-2850) 2927, C=C (1600-1475) 1517, N-H (3500-3100) 3238, C-N (1350-1000) 1217, C=N (1690-1640) 1664. RF value – 0.35

3. Synthesis of 4-(4-(dimethylamino) benzyl)-1-(1-(dimethylamino) propan-2-yl)-3-methyl-1*H*-pyrazol-5(4*H*)-one

^{1}HNMR ($CDCl_3$, 300 MHz)- δ 7.0861(d, J=8.6Hz,2H); 6.689(d, J=9.67Hz,2H); 4.290(dd, J_{HaHc}=10.41Hz,J_{HaHb}=5.64Hz, 1H); 4.093(dd,J_{HbHc}=10.41Hz, J_{HbHa}=5.76Hz, 1H); 3.581(s, 2H); 3.011(dd, J_{HcHa}=12.6Hz, J_{HcHb}=5.97Hz, 1H); 2.904(s,6H); 2.29(s,1H); 2.189(s,6H); 2.117(s, 3H); 1.141(d, J=6.7Hz,3H). Mass (m/e)- Mol. Wt. - 316, (m+1) – 317. IR (Neat) - C=O (1725-1705), C-H (3000-2850) 2929, C=C (1600-1475) 1517, N-H (3500-3100) 3224, C-N (1350-1000) 1346, C=N (1690-1640) 1692. RF value – 0.47

4. Synthesis of 4-(4-(dimethylamino) benzyl)-1-(2-(dimethylamino) ethyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one

^{1}HNMR ($CDCl_3$, 300 MHZ)- δ 7.080(d, J=8.31Hz, 2H); 6.689(d, J=8.34Hz, 2H); 4.316(t, 2H); 3.579(s, 2H); 2.902(s, 6H); 2.741(t, 2H); 2.315(s, 6H); 2.189(s, 1H); 2.092 (s, 3H). Mass (m/e)- Mol. Wt. - 302, (m+1) – 303. IR (Neat) - C=O (1725-1705) 1722, C-H (3150-3050) 3100, C=C (1600-1475) 1517, N-H (3500-3100) 3225, C-N (1350-1000) 1350, C=N (1690-1640) 1666. RF value – 0.52

5. Synthesis of 1-(3-(4-(3-chlorophenyl) piperazin-1-yl) propyl)-4-(4-(dimethylamino) benzyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one

^{1}HNMR ($CDCl_3$, 300 MHz)- δ 7.31 (s, 1H); 7.17(t,1H); 7.09(d, J=9Hz, 1H); 6.88(s,1H); 6.80 (d, J=18Hz, 3H); 6.69(d, J=9Hz, 1H); 4.24(t, J=6Hz, 2H); 3.59(s, 2H); 3.20(s, 6H); 2.60-2.50(m, 8H); 2.35(s, 1H); 2.13(s,3H); 1.99-1.97(m, 2H); 0.90-0.88(m,2H). Mass (m/e)- Mol. Wt. - 467, (m+1) – 468. IR (Neat) - C=O (1725-1705),

C-H (3150-3050) 2926, C=C (1600-1475) 1596, N-H (3500-3100) 3405, C-N (1350-1000) 1221, C=N (1690-1640). RF value – 0.8

6. Synthesis of 4-(4-(dimethylamino) benzyl)-3-methyl-1-(2-morpholinoethyl)-1*H*-pyrazol-5(4*H*)-one

^{1}HNMR ($CDCl_3$, 300 MHz)- δ-7.0482(d,J=8.52Hz,2H); 6.782(d,J=8.58Hz,2H); 4.711(t,2H); 3.812-3.648(m,4H); 3.638(s,2H); 3.489(s,2H); 3.191-3.059(m,4H); 2.926(s,6H); 2.437(s,1H); 1.136(s,3H). Mass (m/e)- Mol. Wt. - 344, (m+1) – 345. IR (Neat) - C=O (1725-1705), C-H (3150-3050) 2997, C=C (1600-1475) 1434, N-H (3500-3100) 3424, C-N (1350-1000) 1315, 1217, C=N (1690-1640) 1640. RF value – 0.5

7. Synthesis of 4-(4-dimethylamino-benzyl)-5-methyl-2-(2-oxiranyl-ethyl)-2, 4-dihydro-pyrazol-3-one

^{1}HNMR ($CDCl_3$, 300 MHz)- δ-6.981(d,J=9Hz,2H); 6.602 (d,J=8.7Hz,2H); 4.215(t,2H); 4.156(d,J=3Hz,1H); 4.110(d, J=2.8Hz, 1H);4.068(d, J=3,1H):3.985(s, 1H); 3.476(d, J=3, 2H); 2.813(s, 6H); 1.999(s, 3H). Mass (m/e)- Mol. Wt. - 287, (m+1) – 288. IR (KBr) - C-O (1300-1000)1223, C-H (3150-3050) 2928, C=C (1600-1475) 1497, N-H (3500-3100) 3484, C-N (1350-1000) 1350, 1116, C=N (1690-1640) 1610. RF value – 0.58.

Antimycobacterial Activity

Tube Agar Microdilution method.

On the basis of literature survey, it was observed that pyrazoline have been reported to show antitubercular activity besides other activities and exhibited good activity against *M. Tuberculosis $H_{37}Rv$* and *M. Tuberculosis $H_{37}Ra$.* The present study was aimed at developing some pyrazoline derivatives and screening them for antitubercular activity.

Antimycobacterial activity of the synthesized compounds was performed by Micro Dilution Method, against *M. Tuberculosis $H_{37}Rv$*. Seven compounds of pyrazoline derivatives were (1-7) synthesized, out of which 3 compounds showed MIC of >12.5 µg/mL, and 2 compounds showed MIC of >25 µg/mL respectively, while 2 compounds displayed MIC of 12 µg/mL. Compound (3) had substituted propyl amine and compound (5) chloro phenyl piperazine propyl amine group at R_2 position of pyrazoline showed a MIC 12 µg/mL, plays a crucial role in antimycobacterial activity as shown in table 1.

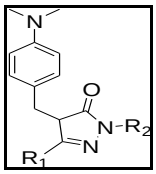
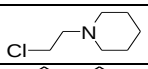
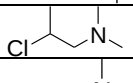
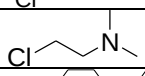
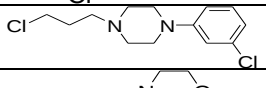
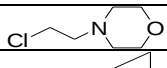


Table-1 Anti-mycobacterium activity of Pyrazoline derivatives

S. No.	R_1	R_2	MIC (µg/mL.)
I.	CH_3		>12.5
II.	CH_3		>12.5
III.	CH_3		12.5
IV.	CH_3		>12.5
V.	CH_3		12.5
VI	CH_3		>25
VII.	CH_3		>25

INH – 0.025(MIC), Rifampicin – 0.20(MIC), Ethambutol – 2.0(MIC)

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