



SYNTHESIS OF SOME NOVEL PYRAZOLINE SCAFFOLDS & THEIR IN-VITRO ANTITUBERCULAR STUDIES

Keerthi Kumar Kodari^{a,#}, O.P.Chourasia^a, N.V. Seelam^b

^aHeterocyclic Research Laboratory, Department of Chemistry, Dr. H. S. Gour Central University, Sagar, M.P, India-470003. Tel: +91-7582264989, E-mail:

keerthikodari@gmail.com

^bOrganic Research Laboratory, Department of Chemistry, K.L.University, Vaddeswaram, Guntur, A.P, India-522502.

Abstract

A new series of 4-benzylidene-1-(2,4-dinitro-phenyl)-3-(4-nitro-phenyl)-5-phenyl-pyrazoline derivatives have been synthesized from the new class of conventional methodology. Further these entities were screened for their antitubercular activity against *M.Tuberculosis H37Rv* and also antimicrobial activity against various class of microorganisms. The final results revealed that most of the tested compounds showed promising activity against all the microorganisms employed. The synthesized compounds were characterized by IR, ¹H NMR, ¹³C NMR and elemental analysis studies.

Keywords

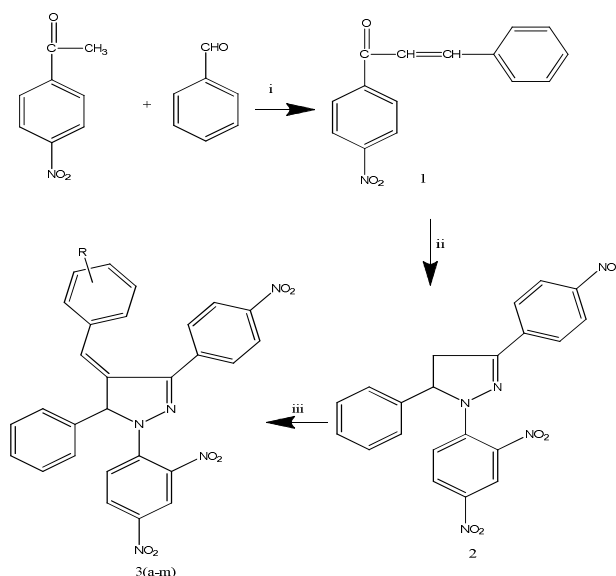
Pyrazolines, Antimycobacterial, Antimicrobial, 2,4-DNP, Ar-CHO

Introduction

Now a day's TB (Tuberculosis) is a major global health problem and is the second leading cause of death from an infectious disease worldwide, by the WHO-2010 report In 2011, there were an estimated 8.5–9.2 million cases and 1.2–1.5 million deaths, by this figure we understand the seriousness of TB and it is a contagious disease for both animals and humans, caused by bacilli belonging the mycobacterium tuberculosis complex, in the majority of the cases TB is due to mycobacterium tuberculosis (koch's bacillus). This disease mainly spread by airborne transmission, this primarily affects on the lungs but it can also affect organs in the central nervous system among others. The disease was called consumption in the past as it would consume from within any one who became infected. In present work all the synthesized compounds were tested in-vitro for their antibacterial, antifungal and antitubercular activity towards different bacterial and fungal strains and *M.Tuberculosis H37Rv* respectively.

A glance at the previous research that indicates that the pyrazoline structures are paying special attention as they belong to class of compounds with proven utility in organic, pharmaceutical and medicinal chemistry. There are number of molecules with five membered rings containing 'N' as a hetero atom. Pyrazoline moiety is an important scaffold known to be reported with several biological aspects including antibacterialⁱ, antifungalⁱⁱ, antiameobatic^{iii-iv}, antitumor^{v-vi}, antiinflammatory^{vii}, anticancer^{viii-ix}, herbicidal^x, anticonvulsant^{xi}, antiviral^{xii},

cytotoxic^{xiii}, antimalarial^{xiv} and antitubercular^{xv-xx} activity. Based on the previous findings it was a thought of interest to synthesize new bioactive pyrazoline derivatives, to improve the activity of pyrazoline derivatives as a potent anti-tubercular active compounds.



Scheme 1 Route of reaction Reaction conditions - i) NaOH, ii) 2,4-DNP, iii) Ar-CHO
 3a-4-OH; 3b-4-OCH₃; 3c-4-Cl; 3d-3,4,5,Tri-OCH₃; 3e-2-OH-3-OCH₃; 3f-N(CH₃)₂; 3g-3-NO₂; 3h-4-NO₂; 3i-4-OH-3-OCH₃; 3j- 2-NO₂; 3k-2-OH; 3l- H; 3m-3-Br.

Results and discussion

To synthesize a new series of condensed Pyrazoline derivatives 2, 4-DNP and different types of aromatic aldehydes were used. After the cyclisation reaction between chalcones and 2, 4-DNP active pyrazoline derivatives were obtained. The resultant compound reacted with various aromatic aldehydes to give chalcone derivatives of pyrazoline moiety. These final structures were confirmed by the IR spectral data with the absorption bands at 3022.41, 3355.18, 3056.85, 1581.65, 1270.24. These were also confirmed by ¹H-NMR and ¹³C-NMR spectral data in the proton positions at 6.2-7.9, 5.03, 7.98 ppm, 58.6, 64.9, 126.8, 128.2, 130.6, 135.2, 148.8, 152.9 respectively. The resultant final products were tested against standard strains which were produced from the microbial type collection (MTCC) and gene bank (Institute of microbial technology, Chandigarh, India). Different type of Gram-positive, Gram-negative bacteria and fungi were used. The selected strains for anti-bacterial activity were *B. thuringiensis* (MTCC No: 4714), *B. subtilis* (MTCC 441), *E. coli* (MTCC 443), *P. aeruginosa* (MTCC 1688) and the following fungal strains, *C. albicans* (MTCC 227), *A. fumigatus* (MTCC 3008), *F. oxysporum* (MTCC No: 7392) were used. The results were compared with standard drugs streptomycin for antibacterial activity and treflucan for antifungal activity.

Biology

According to the obtained results the synthesized Pyrazoline derivatives shows prominent antimicrobial activity. The synthesized Pyrazoline derivatives had shown promising antimicrobial activity on comparison with the controls, the compounds 3c, 3f, 3g, 3h, 3j, 3k were shown good activity against *B. subtilis*, compounds 3c, 3g, 3h, 3j are shown promising activity against *B. thuringiensis*, compounds 3c, 3f, 3h, 3j are potent against *P. aeruginosa*. 3c, 3f, 3g, 3h, 3j these compounds are active against *E.Coli*. By the overall comparison of the

results the derivatives 3c, 3h, 3j potent against selected bacteria. On overall comparison of the antimicrobial data, the chloro substituted derivative compound '3c' was highly potent against all bacterial strains on rest of all other derivatives. At the same time the different nitro substituted derivatives have also shown good activity against *Bacillus Subtilis*, than rest of all but the 2-NO₂ derivative is highly active against *Bacillus Thuringiensis*. The remaining derivatives have shown moderate to less activity. For antifungal activity the compounds 3a, 3b, 3d, 3e, 3l have shown good antifungal activity. The compound '3d' (3,4,5-trimethoxy) is highly active against all the fungal strains, the results have been showed in **Table I**. By this comparison the selected derivatives have taken further steps (anti- tubercular activity).

Table I Antibacterial And Antifungal Activities of Synthesized compounds (3a-m). MIC (µg/mL)

Comp.code	R	Antibacterial activity				Antifungal activity		
		B.subt	B.thur	E.coli	P.aeru	A.fumig	F.oxys	C.albica
3a	4-OH	25	25	50	>50	3.125	6.25	3.125
3b	4-OCH ₃	12.5	25	-	25	6.25	3.125	3.125
3c	4-Cl	<3.125	3.125	3.125	3.125	>50	25	-
3d	3,4,5, Tri-OCH ₃	25	25	25	>50	3.125	3.125	<3.125
3e	2-OH-3-OCH ₃	25	>50	-	-	3.125	6.25	3.125
3f	N(CH ₃) ₂	3.125	6.25	3.125	3.125	25	>50	12.5
3g	3-NO ₂	3.125	3.125	3.125	6.25	-	25	25
3h	4-NO ₂	<3.125	3.125	3.125	3.125	12.5	25	25
3i	4-OH-3-OCH ₃	50	-	>50	-	12.5	6.25	6.25
3j	2-NO ₂	<3.125	1.562	3.125	3.125	25	25	>50
3k	2-OH	25	50	50	>50	6.25	3.125	3.125
3l	H	12.5	-	12.5	-	25	25	12.5
3m	3-Br	3.125	6.25	6.25	6.25	25	-	25
Streptomycin	-	3.125	6.25	6.25	6.25	-	-	-
Treflucan	-	-	-	-	-	3.125	3.125	3.125

B. subtilis (MTCC 441), *B. thuringiensis* (MTCC No: 4714), *E. coli* (MTCC 443), *P. aeruginosa* (MTCC 1688), and the following fungal strains *A. fumigatus* (MTCC 3008), *F. oxysporum* (MTCC No: 7392), *C. albicans* (MTCC 227).

Antitubercular activity

The antitubercular activities of the synthesized compounds were screened against *Mycobacterium Tuberculosis* H37Rv^{xxi} Isoniazid as a standard. Generally the compounds which having electron-withdrawing groups showed good anti tubercular activity, and the compounds 3c, 3e, 3f, 3j have shown good activity against the test organism the results are summarized in **Table II**.

Table II antimycobacterial activity of synthesized compounds			
Comp.code	MIC(μ g/ml)	Comp.code	MIC(μ g/ml)
3a	>25	3h	25
3b	>25	3i	12.50
3c	6.25	3j	>3.125
3d	12.50	3k	>12.50
3e	6.25	3l	>25
3f	6.25	3m	>12.50
3g	>25	<i>Isoniazid</i>	4

Experimental

Material and Methods :

All the melting points of the synthesized compounds were determined in open capillaries and are uncorrected. IR spectra were recorded for KBr disc on Shimadzu-8400 FT-IR spectrophotometer. ^1H and ^{13}C NMR spectra were measured on a Bruker Avance-II 400 spectrometer operating at 400, 100.6 MHz respectively. Chemical shifts (δ) are reported in ppm and TMS is used as an internal standard. Purity of the compounds checked by TLC on silica gel 'G' plates, plates were visualizing with ultraviolet light (or) iodine.

General procedure for preparation of 1-(4-Nitrophenyl)-3-phenylpropane. (1)

An equimolar mixture of p-nitro acetophenone and benzaldehyde was taken in a round bottomed flask, with 15ml of ethanol and the mixture was stirred for 15 min, then 20ml of 20%NaOH solution was added drop by drop with continuous stirring to the resultant mixture, after the stirring left for overnight, then acidified with dil HCl and poured it into crushed ice, finally the solid obtained was filtered and crystallized. The progress of the reaction was checked by TLC. The compound was confirmed by IR spectral data 3038.44 (C-H in Ar), 1554.63(N-O), 3055.16 (C-H in ring).

General procedure for preparation of 1-(2,4-Dinitrophenyl)-3-(4-nitrophenyl)-5-phenyl-4,5-dihydro-pyrazoline (2)

An equimolar mixture of (1) (0.01 mol) and 2,4-Dinitrophenylhydrazine (0.01 mol) were dissolved in 15ml glacial acetic acid, the mixture was refluxed with continuous stirring up to 5-6 hours. The progress of the reaction was checked by TLC. After completion of the reaction the mixture was cooled then poured it in to crushed ice finally the solid obtained was filtered and crystallized. This compound was confirmed by IR spectral data studies 3022.41 (C-H in Ar), 3355.18(O-H), 3056.85 (C-H in ring), 1581.65 (C=N), 1270.24 (C-N).

General procedure for preparation of (Z)-4-Benzylidene-1-(2,4-dinitrophenyl)-3-(4-nitrophenyl)-5-phenyl-4,5-dihydro-1H-pyrazole 3(a-m)

A mixture of 1-(2,4-Dinitrophenyl)-3-(4-nitrophenyl)-5-phenyl-4,5-dihydro-pyrazoline (0.01mol), aromatic aldehyde (0.01mol) and (0.01mol) of anhydrous sodium acetate was dissolved in 12ml of DMF and stirring it with continuous reflux up to 8hours the progress of the reaction was checked by TLC. After completion of the reaction the reaction mixture was cooled and then poured it in to crush ice finally the solid obtained was filtered and crystallized. The physical and analytical data of synthesized derivatives 3a-m were depicted in Table III.

Comp. code	Formula	M.P(°c)	Yield(%)	Analysis (calc. / found) (%)					
				C		H		N	
3a	C ₂₈ H ₁₉ N ₅ O ₇	210	56.7	62.57	62.43	3.56	3.41	13.03	12.90
3b	C ₂₉ H ₂₁ N ₅ O ₇	202	63.1	63.16	63.02	3.84	3.67	12.70	12.59
3c	C ₂₈ H ₁₈ ClN ₅ O ₆	234	75.2	60.49	60.40	3.26	3.21	12.60	12.53
3d	C ₃₁ H ₂₅ N ₅ O ₉	263	54.8	60.88	60.72	4.12	4.01	11.45	11.31
3e	C ₂₉ H ₂₁ N ₅ O ₈	213	55.4	61.38	61.20	3.73	3.56	12.34	12.22
3f	C ₃₀ H ₂₄ N ₆ O ₆	221	52.6	63.82	63.68	4.28	4.12	14.89	14.68
3g	C ₂₈ H ₁₈ N ₆ O ₈	232	68.2	59.37	59.22	3.20	3.04	14.84	14.65
3h	C ₂₈ H ₁₈ N ₆ O ₈	226	66.4	59.37	59.30	3.20	3.15	14.84	14.76
3i	C ₂₉ H ₂₁ N ₅ O ₈	206	56.3	61.38	61.25	3.73	3.61	12.34	12.27
3j	C ₂₈ H ₁₈ N ₆ O ₈	239	76.3	59.37	59.22	3.20	3.06	14.84	14.69
3k	C ₂₈ H ₁₉ N ₅ O ₇	198	54.3	62.57	62.44	3.56	3.41	13.03	12.89
3l	C ₂₈ H ₁₉ N ₅ O ₆	201	52.9	64.49	64.37	3.67	3.54	13.43	13.31
3m	C ₂₈ H ₁₈ BrN ₅ O ₆	256	61.6	56.1	55.84	3.02	2.90	11.66	11.51

Table 2 Here (Z)-4-((1-(2,4-dinitrophenyl)-3-(4-nitrophenyl)-5-phenyl-1H-pyrazol-4(5H)-ylidene) methyl)phenol. (3a)

IR (KBr) cm⁻¹: 3029.41 (C-H in Ar), 3345.18(O-H), 3056.85 (C-H in ring), 1590.19 (C=N), 1278.03 (C-N). ¹H NMR (400MHz, DMSO-d₆) δ ppm: 7.95 (s, 1H, =CH), 5.01 (s, 1H, C-H), 6.91-7.93 (m, 16H, Ar-H), 5.41 (s, 1H, O-H); ¹³C NMR (100MHz, DMSO-d₆) δ ppm: 51.6, 63.2, 116.1, 128.5, 129.4, 130.1, 134.5, 148.7, 152.7, 153.5.

(Z)-1-(2,4-dinitrophenyl)-4-(4-methoxybenzylidene)-3-(4-nitrophenyl)-5 phenyl-4,5-dihydro-1H-pyrazole.3b

IR (KBr) cm⁻¹: 3029.41 (C-H in Ar), 1241.23(C-O-C), 3061.27 (C-H in ring), 1590.19 (C=N), 1256.49 (C-N). ¹H NMR (400MHz, DMSO-d₆) δ ppm: 7.93 (s, 1H, CH=), 5.03 (s, 1H, C-H), 7.01-7.81 (m, 16H, Ar-H), 3.76 (s, 3H, OCH₃); ¹³C NMR (100MHz, DMSO-d₆) δ ppm: 54.1, 58, 65, 114.1, 129.5, 134.5, 148.1, 160.7.

(Z)-4-(4-chlorobenzylidene)-1-(2,4-dinitrophenyl)-3-(4-nitrophenyl)-5-phenyl-4,5-dihydro-1H-pyrazole.3c

IR (KBr) cm⁻¹: 3031.41 (C-H in Ar), 1509.16(N-O), 3056.85 (C-H in ring), 1591.42 (C=N), 1261.73 (C-N), 1093.54 (C-Cl); ¹H NMR (400MHz, DMSO-d₆) δ ppm: 7.21-7.85 (m, 16H, Ar-H), 7.95 (s, 1H, =CH), 5.0 (s, 1H, C-H); ¹³C NMR (100MHz, DMSO-d₆) δ ppm: 59.1, 63.6, 126.5, 128.8, 129.4, 134.5, 148.5, 151.6.

(Z)-1-(2,4-dinitrophenyl)-3-(4-nitrophenyl)-5-phenyl-4(3,4,5-trimethoxybenzylidene)-4,5-dihydro-1H-pyrazole.3d

IR (KBr) cm⁻¹: 3029.27 (C-H in Ar), 1541.03(N-O), 3058.39 (C-H in ring), 1586.08 (C=N), 1279.51 (C-N), 1237.91 (C-O-C), 2941.16 (C-H in CH₃); ¹H NMR (400MHz, DMSO-d₆) δ ppm: 7.35-7.89 (m, 14H, Ar-H), 7.95 (s, 1H, =CH), 4.98 (s, 1H, CH), 3.91(s, 6H, OCH₃), 3.78(s, 3H, OCH₃); ¹³C NMR (100MHz, DMSO-d₆) δ ppm: 58.3, 60.9, 71.3, 114.7, 119.7, 123.1, 129.5, 136.7, 148.8, 157.3, 160.3.

(Z)-2-((1-(2,4-dinitrophenyl)-3-(4-nitrophenyl)-5-phenyl-1H-pyrazol-4(5H)-ylidene) methyl)-6-methoxyphenol.3e

IR (KBr) cm^{-1} : 3318.79(O-H), 3031.57 (C-H in Ar), 1518.03(N-O), 3056.18 (C-H in ring), 1590.21 (C=N), 1278.03 (C-N), 2938.06 (C-H), 1219.57 (C-O-C) ; ^1H NMR (400MHz, DMSO- d_6) δ ppm: 6.9 -7.8 (m, 15H, Ar-H), 7.93 (s, 1H, =CH), 4.98 (s, 1H, C-H), 5.47 (s, 1H, OH), 3.89 (s, 3H, OCH₃); ^{13}C NMR (100MHz, DMSO- d_6) δ ppm: 55.3, 58.6, 61.9, 123.1, 126.7, 128.4, 129.5, 138.1, 146.7, 160.3.

(Z)-4-((1-(2,4-dinitrophenyl)-3-(4-nitrophenyl)-5-phenyl-1H-pyrazol-4(5H)-ylidene) methyl)-N,N-dimethylaniline.3f

IR (KBr) cm^{-1} : 3038.57 (C-H in Ar), 1518.18(N-O), 3056.18 (C-H in ring), 1591.86 (C=N), 1281.93 (C-N), 2951.49 (C-H); ^1H NMR (400MHz, DMSO- d_6) δ ppm: 6.8 -7.81 (m, 16H, Ar-H), 7.93 (s, 1H, =CH), 5.08 (s, 1H, C-H), 2.90 (s, 6H, OCH₃); ^{13}C NMR (100MHz, DMSO- d_6) δ ppm: 42.7, 60.8, 70.1, 113.61, 117.01, 126.1, 128.3, 129.5, 134.5, 148.3, 150.1, 158.1.

(Z)-1-(2,4-dinitrophenyl)-4-(3-nitrobenzylidene)-3-(4-nitrophenyl)-5-phenyl-4,5-dihydro-1H-pyrazole.3g

IR (KBr) cm^{-1} : 3031.16 (C-H in Ar), 1547.18(N-O), 3057.34 (C-H in ring), 1593.27 (C=N), 1263.51 (C-N); ^1H NMR (400MHz, DMSO- d_6) δ ppm: 7.48 -7.81 (m, 16H, Ar-H), 7.92 (s, 1H, =CH), 4.96 (s, 1H, C-H) ; ^{13}C NMR (100MHz, DMSO- d_6) δ ppm: 58.6, 68.1, 123.0, 126.5, 128.4, 129.5, 135.1, 148.7, 151.6.

(Z)-1-(2,4-dinitrophenyl)-4-(4-nitrobenzylidene)-3-(4-nitrophenyl)-5-phenyl-4,5-dihydro-1H-pyrazole.3h

IR (KBr) cm^{-1} : 3057.34 (C-H in ring), 3031.16 (C-H in Ar), 1547.93(N-O), 1596.18 (C=N), 1269.18 (C-N); ^1H NMR (400MHz, DMSO- d_6) δ ppm: 7.2 -7.8 (m, 16H, Ar-H), 7.98 (s, 1H, =CH), 5.04 (s, 1H, C-H) ; ^{13}C NMR (100MHz, DMSO- d_6) δ ppm: 58.6, 68.1, 123.6, 126.5, 128.3, 129.5, 135.1, 148.7, 153.07.

(Z)-2-((1-(2,4-dinitrophenyl)-3-(4-nitrophenyl)-5-phenyl-1H-pyrazol-4(5H)-ylidene) methyl)-4-methoxyphenol.3i

IR (KBr) cm^{-1} : 3361.27(O-H), 3043.57 (C-H in Ar), 1541.26 (N-O), 3059.73 (C-H in ring), 2947.03 (C-H), 1590.61 (C=N), 1271.38 (C-N), 1238.57 (C-O-C) ; ^1H NMR (400MHz, DMSO- d_6) δ ppm: 6.98 -7.84 (m, 15H, Ar-H), 7.98 (s, 1H, =CH), 5.06 (s, 1H, C-H), 5.58 (s, 1H, OH), 3.86 (s, 3H, OCH₃); ^{13}C NMR (100MHz, DMSO- d_6) δ ppm: 59.6, 71.3, 114.6, 117.5, 123.1, 128.4, 129.5, 130.6, 134.5, 148.1, 153.6, 157.2, 160.3.

(Z)-1-(2,4-dinitrophenyl)-4-(2-nitrobenzylidene)-3-(4-nitrophenyl)-5-phenyl-4,5-dihydro-1H-pyrazole.3j

IR (KBr) cm^{-1} : 3056.34 (C-H in ring), 3038.16 (C-H in Ar), 1543.16(N-O), 1581.47 (C=N), 1268.54 (C-N); ^1H NMR (400MHz, DMSO- d_6) δ ppm: 7.0 -7.7 (m, 16H, Ar-H), 7.95 (s, 1H, =CH), 5.03 (s, 1H, C-H) ; ^{13}C NMR (100MHz, DMSO- d_6) δ ppm: 59.6, 69.1, 123.7, 126.1, 126.4, 128.9, 129.5, 135.3, 148.7, 157.01.

(Z)-2-((1-(2,4-dinitrophenyl)-3-(4-nitrophenyl)-5-phenyl-1H-pyrazol-4(5H)-ylidene) methyl)phenol.3k

IR (KBr) cm^{-1} : 3039.18 (C-H in Ar), 3328.29(O-H), 3063.07 (C-H in ring), 1573.56 (C=N), 1281.16 (C-N), 1543.11 (N-O). ^1H NMR (400MHz, DMSO- d_6) δ ppm: 6.98-7.78 (m, 16H, Ar-H), 5.01 (s, 1H, C-H), 7.95 (s, 1H, =CH), 5.58 (s, 1H, OH); ^{13}C NMR (100MHz, DMSO- d_6) δ ppm: 58.6, 69.1, 116.3, 121.1, 123.6, 128.6, 130.1, 135.5, 148.7, 153.1.

(Z)-4-Benzylidene-1-(2,4-dinitrophenyl)-3-(4-nitrophenyl)-5-phenyl-4,5-dihydro-1H-pyrazole.3l

IR (KBr) cm^{-1} : 3056.01 (C-H in ring), 3029.23 (C-H in Ar), 1549.81 (N-O), 1595.26 (C=N), 1278.03 (C-N). ^1H NMR (400MHz, DMSO- d_6) δ ppm: 6.2-7.9 (m, 17H, Ar-H), 5.03 (s, 1H, C-H), 7.98 (s, 1H, =CH); ^{13}C NMR (100MHz, DMSO- d_6) δ ppm: 59.1, 68.3, 123.1, 126.3, 128.4, 129.5, 134.6, 147.1, 157.1.

(Z)-4-(4-bromobenzylidene)-1-(2,4-dinitrophenyl)-3-(4-nitrophenyl)-5-phenyl-4,5-dihydro-1H-pyrazole.3m

IR (KBr) cm^{-1} : 3030.68 (C-H in Ar), 1536.44(N-O), 3056.85 (C-H in ring), 1591.32 (C=N), 1259.19 (C-N), 1082.54 (C-Br); ^1H NMR (400MHz, DMSO- d_6) δ ppm: 7.24-7.8 (m, 16H, Ar-H), 7.91 (s, 1H, =CH), 5.06 (s, 1H, C-H); ^{13}C NMR (100MHz, DMSO- d_6) δ ppm: 58.6, 64.9, 126.8, 128.2, 130.6, 135.2, 148.8, 152.9.

Antitubercular Activity Procedure

All the synthesized compounds of series 3a-m were evaluated for their antitubercular activity. Drug susceptibility and determination of MIC of the test compounds against M. tuberculosis H37Rv were performed by agar micro dilution method, where two fold dilutions of each test compound were added into 7H10 agars supplemented with OADC and organism. A culture of used microorganism M. tuberculosis H37Rv growing on L-J medium was harvested in 0.85% saline with 0.05% Tween-80. A suspension of compounds was prepared in DMSO. This suspension was added to (in tubes) 7H10 middle brook's medium (containing 1.7 mL medium and 0.2 mL OADC supplement) at different concentrations of compound keeping the volume constant, that is, 0.1 mL medium was allowed to cool keeping the tubes in slanting position. These tubes were then incubated at 37°C for 24hours followed by streaking of M. tuberculosis H37Rv (5×10^4 bacilli per tube). These tubes were then incubated at 37 °C. Growth of bacilli was seen after 30 days of incubation. Tubes having the compounds were controlled with control tubes where medium alone was incubated with H37Rv. The concentration at which complete inhibition of colonies occurred was taken as active concentration of test compound. Isoniazid was used as standard drug. The MIC levels of compounds 3a-m against these organisms were given in **Table II**.

Conclusion

The derived pyrazoline analogues were screened for their antitubercular and antimicrobial studies. The following conclusion was drawn, after the biological screening of synthesized compounds, due to the presence of 4-Cl and 2-NO₂ groups the title compound shows promising antitubercular and antimicrobial activity rest of all.

Acknowledgements

The authors are grateful to The Head, Department of Chemistry, Dr. H. S. Gour University Sagar for providing necessary facilities to carry out this work. We are thanking full to The Director Micro care laboratory Surat, for providing anti-microbial & anti tubercular data. We are grateful to The Director, SAIF, Panjab University for providing spectral data.

References

- i. A.Davood, S.Maseud, *Molecules*.7, 885(2002).
- ii. N.Gautam, O.P.Chourasia, *Indian. J. Chem*, 49(B), 830(2010).
- iii. A.Mohammad, R.B.Abdul, A.Fareeda, A.Amir, *Eur. J. Med. Chem*, 44, 417(2009).
- iv. R.B.Abdul, A.Fareeda, A.Amir, *Eur. J. Med. Chem*, 44, 426(2009).
- v. A.F.R. Sherif, *Bio. Org & Med. Chem*, 14, 6475(2006).

- vi.** C.Cenzo, O.Valentina, V.Loredana, C.Massimo, P.Claudio, *Bio. Org & Med. Chem*, 18, 6238(2010).
- vii.** K.S.Pawan, K.Satish, K.Pawan, K.Dhirender, D.Yogita, R.A.Kamal, *Eur. J. Med. Chem*, 45, 2650(2010).
- viii.** C.Li-Chen, H.Li-Jiau, H.Mei-Hua, F.Mei-Chi, Y.Jai-Sing, Z.Shi-Hong, L.Hui-Yi, L.Fang-Yu, T.Che-Ming, K.Sheng-Chu, *Eur. J. Med. Chem*, 45, 1395(2010).
- ix.** B.Sameena, J.Kalim, A.Shamim, I.G.Rathish, S.Surender, M.S.Alam, *Eur. J. Med. Chem*, 46, 5763(2011).
- x.** K.M.Moimoto, K.Makino, S.Yamamoto, G.Sakoto, *J. Heter. Chem*, 27,807(1990).
- xi.** N.Gokhan, A.Yesxilada, G.Ucar, K.Erol, A.A.Bilgin. *Arch. Pharm. Pharm. Med. Chem.*;336, 362(2003).
- xii.** IEl-S.Osama, M.B.Mohamed, M.I.Samy, P.Christophe, A.Graciela, S.Robert, B.Jan, A.R.Adel, *Eur. J. Med. Chem*, 44, 3746(2009).
- xiii.** S.Y.Mohammad, A.S.Anees, A.A.Mohamed, M.Vedigounder, C.Raghu J. Of. The. *Chin. Chem Soc*, 54, 81(2007).
- xiv.** N.A.Badri, S.Deepika, T.Mugdha, K.S.Asish, G.Ramarao, B.Saroj, P.K.Mahabir, *Eur. J. Med. Chem*, 45, 430(2010).
- xv.** E.A.S.Pedro, F.R.Daniela, G.B.Helio, I.I.Agustina, R.O.Marli, C.Tatiane, N.Jussara, R.M.Hector, Z.Nilo, A.P.M.Marcos. *Int. J. Ant. Agents*, 32, 139(2008).
- xvi.** A.A.Mohamed, S.Mohammad, A.S.Anees, *Eur. J. Med. Chem*, 42, 268(2007).
- xvii.** T .Tasneem, R.K.Ravindra, T.M.Gireesh, K.H.Raveendra, B.M.Sheetal, *Eur. J. Med. Chem*, 46, 4366(2011).
- xviii.** R.C.Khunt, V.M.Khedkar, R.S.Chawda, N.A.Chauhan, A.R.Parikh, E.C.Coutinho, *Bio Org. Med. Chem. Lett*,22,666(2012).
- xix.** J.A.Mohamed, G.S.Jeyabalan, B.J.Chandra, R.D.Kunduri, K.Habibullah, N.M.Shivli, *Bio.Org & Med. Chem. Lett*, 22, 969(2012).
- xx.** Z.Daniele, G.M.Maria, L.Erik, S.Giuditta, B.Elena, V.Luciano, *Bio.Org & Med Chem*,16, 4516(2008).
- xxi.** S.Nareshvarma, S.P.Shrivastava. *Bull. Kor. Chem. Soc*, 32, 3996(2011).

Received on August7, 2014.