International Journal of Advanced Research in Chemical Science (IJARCS)
Volume 2, Issue 12, December 2015, PP 36- 41
ISSN 2349-039X (Print) & ISSN 2349-0403 (Online)
www.arcjournals.org

Synthesis and Evaluation of Antimicrobial Activities of Some New Pyrazoline Derivatives

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Abstract: *A series of N-formyl pyrazolines (4a-l) were prepared by treating 1,3-diarylpropenones(3a-l)with hydrazine hydrate and acetic acid at reflux condition. 1,3-diarylpropenones (3a-k) were obtained by treating 4-methylthio benzaldehyde and arylketone in the presence of 10% NaOH and ethanol. The structures of newly synthesized compounds are characterized by elemental analysis, FT-IR, ^{1}H-NMR and mass spectroscopic studies and screened for their antimicrobial activities. The preliminary results revealed that some of the compounds exhibited promising antimicrobial activities.*

Keywords: *Antibacterial, Antifungal, Propenones and N-formyl pyrazolines*

1. INTRODUCTION

Pyrazoline derivatives constitute an important class of organic compounds with diverse chemical1 and pharmacological applications[1] and therefore they are useful in drug research.

They exhibit a broad spectrum of biological activities such as antimicrobial[2-4], antifungal[5-8], antiviral[9,10], antiamoebic[11,12], anti-HIV[13], tyrosinase inhibitors[14], mollucidal[15], Antidepressant[16], antidiabetic[17], anti-inflammatory[18], Anticonvulsant agents[19], Hypotensive activity[20], Antioxidant activity[21]. In view of the importance of pyrazoline derivatives as revealed by the literature and in continuation of our work on pyrazlines we decided to synthesize pyrazolines baering 4-Methylthiophenyl moiety. Their characterization and antimicrobial activities are discussed herein.

2. EXPERIMENTAL

The newly synthesized compounds were confirmed by their spectral analysis. The chemicals used for the synthesis of novel pyrazolines were of standard quality. 4-Methylthiobenzaldehyde, different arylketones, formic acid and hydrazine hydrate were procured from Sigma-Aldrich, Bengaluru, India. Potassium hydroxide from Spectrochem, Mumbai-India. Melting points were determined in an open capillary tube and are uncorrected. IR spectra were recorded on a SHIMADZU-FTIR Infrared Spectrometer in KBr (ν_{max} in cm^{-1}). ^{1}H-NMR spectra were recorded in $CDCl_3$ on amx (400 MHz) spectrometer using TMS as internal standard. Elemental analysis was carried out on a Euro-E-300, FABMS spectra were recorded on a JEOL SX 102/DA-6000 Mass Spectrometer using argon/xenon (6kv, 10mA). Completion of the reaction was monitored by thin layer chromatography (TLC) using Merck silica gel 60 F_{254} coated alumina plates. The synthetic pathway is presented in **reaction scheme**. The data of the novel pyrazoline derivatives are presented in **Tables 1** and the biological activity data are tabulated in **Tables 2** and **3**.

2.1. Reaction Scheme

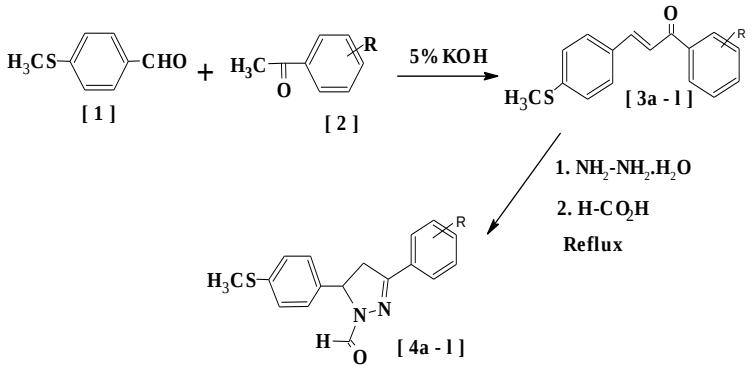

Table1. *Characterization data of N-formyl pyrazolines (4a-l)*

Compd	R_1	MF	M.W	M.P(^{0}C)	Analysis (%)Found (Calculated)		
					C	H	N
4a	4-CH_3	$C_{18}H_{18}N_2OS$	310.00	158-160	69.71 69.67	5.84 5.80	9.06 9.00
4b	4-OCH_3	$C_{18}H_{18}N_2O_2S$	326.00	115-118	66.32 66.25	5.59 5.52	8.62 8.58
4c	4-Cl	$C_{17}H_{15}N_2OSCl$	330.45	176-179	61.66 61.73	4.48 4.53	8.38 8.47
4d	4-Br	$C_{17}H_{15}N_2OSBr$	374.99	194-198	54.49 54.40	4.16 4.00	7.54 7.46
4e	4-H	$C_{17}H_{16}N_2OS$	296.00	166-164	68.84 68.91	5.52 5.40	9.57 9.45
4f	4-NO_2	$C_{17}H_{15}N_3O_3S$	341.00	148-151	59.73 59.82	4.53 4.40	12.25 12.31
4g	4-F	$C_{17}H_{15}N_2OSF$	314.00	170-173	64.74 64.96	4.89 4.77	8.97 8.85
4h	4-SCH_3	$C_{17}H_{16}N_2OS_2$	342.00	155-159	63.23 63.15	5.39 5.26	8.31 8.18
4i	2,4-Cl_2	$C_{17}H_{14}N_2OSCl_2$	364.90	146-151	56.09 55.90	3.77 3.83	7.76 7.67
4j	2,3,4-Cl_3	$C_{17}H_{13}N_2OSCl_3$	399.35	174-187	51.22 51.08	3.38 3.25	7.89 7.01
4k	2,4-Cl_2 -5-F	$C_{17}H_{13}N_2O$ SCl_2F	382.90	202-205	53.27 53.13	3.52 3.39	7.42 7.31
4l	4-C_6H_5	$C_{23}H_{20}N_2OS$	372.00	210-215	74.31 74.19	5.54 5.37	7.67 7.52

2.2. 3-(aryl)-1-[4-(methylthio)phenyl]prop-2-en-1-ones were prepared as per the procedure reported in the literature22, 23,24 (3a-l)

2.3. General Procedure for the synthesis of pyrazolines (4a-l)

Hydrazine hydrate (90%, 5 ml) was added dropwise to a mixture of propenone **(3)** (10 mmol) and formic acid (25 ml). The reaction mixture was heated under reflux for four hours, then cooled and poured on to crushed ice. The resulting pyrazolines were collected by filtration and recrystallized from a mixture of dimethylformamide and ethanol. The characterization data of pyrazolines are given in **Table 1.**

4a: **IR(KBr, cm^{-1}):** 3058(Ar-H), 2891(C-H of CH_3), 1651(N-CHO) 1592, 1495 and 1428(C=N, C=C); **^{1}HNMR($CDCl_3$):** δ 2.39 (s, 3H, CH_3), 2.47 (s, 3H, SCH_3), 3.93(dd, 2H, *J* = 4.8 Hz), 3.14(dd, 2H, *J*=4.8 Hz), 3.78(dd, 2H, *J*=11.6 Hz), 3.74(d, 1H, *J*=11.6 Hz), 7.62(d, 2H, *J*=8 Hz, 4-methylthiophenyl), 7.23(d, 2H, *J*=8 Hz, 4-methylthiophenyl), 7.16(d, 2H, *J*=8.4 Hz, 4-methylphenyl), 7.21(d, 2H, *J*=8.4 Hz, 4-methylphenyl); **FAB MS (*m/z*, %):** 310(M^+, 89), 289(70), 225 (39), 165 (26).

4b: **IR(KBr, cm^{-1}):** 3043(Ar-H), 2864(C-H, OCH_3), 1659(N-CHO) 1602, 1588 and 1428(C=N, C=C); **^{1}HNMR($CDCl_3$):** δ 2.43 (s, 3H, SCH_3), 3.84(s, 3H, OCH_3), 3.12-3.17(dd, 2H, *J*=4.8 Hz),

3.72-3.76(dd, 2H, *J* =4.8 Hz), 5.44-5.47(dd, 1H, *J*=11.6 Hz), 6.93(d, 2H, *J*=8.8 Hz, 4-methoxyphenyl), 7.66(d, 2H, *J*=8.8Hz, 4-methoxyphenyl), 7.16(d, 2H, *J*=8.4, 4-methylthiophenyl), 7.20(d, 2H, *J*=8.4, 4-methylthiophenyl), 8.91(s, 1H, CHO) ; **FAB MS (*m/z*, %):** 326 (M^+, 69), 289(54), 281(47).

4c: **IR(KBr, cm^{-1}):** 3059(Ar-H), 2889 (C-H 0f CH_3), 1651(N-CHO), 1595, 1496 and 1428 (C=C, C=N); **^{1}HNMR($CDCl_3$):** δ 2.44(s, 3H, SCH_3), 3.18(dd, 2H, *J*=4.8 Hz), 3.13(d, 1H, *J*=4.8 Hz), 3.78(d, 1H, *J*=11.6 Hz), 3.74(d, 1H, *J*=12Hz), 7.66(d, 2H, *J*=8.8 Hz, 4-chlorophenyl), 7.22(d, 2H, *J*=8 Hz, 4-chlorophenyl), 7.40(d, 2H, *J*=8.4 4-methylthiophenyl), 7.16(d, 2H, *J*=8.4, 4-methylthiophenyl); **FAB MS (*m/z*, %):** 330(M^+, 78), 331(M^++1, 100), 332(M+2, 51), 289(65), 279(42), 167(23).

4d: **IR(KBr, cm^{-1}):** 3058(Ar-H), 2891(C-H, SCH_3), 1651(N-CHO), 1592 and 1495(C=N, C=C); **^{1}HNMR($CDCl_3$):** δ 2.44(s, 3H, SCH_3), 3.13-3.18(dd, 2H, *J*=4.8 Hz and *J =5.2*), 3.74-3.78(dd, 2H, *J*=12 Hz), 5.48-5.51(dd, 1H, *J*=4.8 Hz and *J*=5.2 Hz), 7.54(d, 2H, *J*=4 Hz, 4-bromophenyl), 7.57(d, 2H, *J*=5.2 Hz, 4-bromophenyl), 7.16(d, 2H, *J*=8.4 Hz, 4-methylthiophenyl), 7.22(d, 2H, *J*=8.4 Hz, 4-methylthiophenyl), 8.93(s, 1H, CHO); **FAB MS(*m/z*, %):** 375(M^+, 64), 377(M+1, 61), 289(25), 281(22).

4l: **IR(KBr, cm^{-1}):** 3031(Ar-H), 2916(C-H, SCH_3), 1658(N-CHO), 1586 and 1601(C=N, C=C); **^{1}HNMR($CDCl_3$):** δ 2.47(s, 3H, SCH_3), 3.240-3.24(dd, 2H, *J*=4.8 Hz), 3.79-3.83(dd, 2H, *J*=11.6 Hz), 5.52-5.49(dd, 1H, *J*=4.8 Hz), 7.80(d, 2H, *J*=8 Hz, 4-methylthiophenyl), 7.61(d, 2H, *J*=8 Hz, 4-methylthiophenyl), 7.17-7.73(m, 9H, 4-phenyl), 8.10(s, 1H, CHO); **FAB MS(*m/z*, %):** 372(M^+,72), 318(86), 289(63), 279(49), 167(28).

3. BIOLOGICAL ACTIVITY

3.1. Antibacterial activity

The newly synthesized compounds **(4a-l)** were screened for their antibacterial activities against *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25922) *Pseudomonas aeruginosa (ATTC-27853)* and *Klebsiella pneumoniae* bacterial strains by the disc diffusion method[25, 26]. Discs measuring 6.25 mm in diameter were punched from Whatman no.1 filter paper. Batches of 100 discs were dispensed to each screw capped bottles and sterilized by dry heat at 140 ^{0}C for an hour. The test compounds were prepared with different concentrations using *N, N* dimethyl formamide. Exactly 1 mL containing 100 times the amount of chemical in each disc was added to each bottle, which contains 100 discs. The discs of each concentration were placed in nutrient agar medium inoculated with fresh bacterial species separately. The plates were incubated at 37^{o}C for 24h. Ciprofloxacin was used as a standard drug. Solvent and growth controls were kept separately and the zone of inhibition (in mm) was measured and the antibacterial activity was determined by measuring the diameter of inhibition zone. The results of such studies are given in **Table 2.**

3.2. Antifungal activity

All the newly synthesized compounds **(4a-l)** were screened for their antifungal activity against *Candida Albicans* (NICM No.300*), Aspergillus Fumigatus* (NICM No.902), *Aspergillus Flavus* (NICM No.524) *and Trichophyton mentagrophytes* (recultured) in DMSO by serial plate dilution method[27, 28]. Sabourauds agar (prepared by dissolving peptone (1g), D-glucose (4g) and agar (2g) in distilled water (100 ml) and adjusting the pH to 5.7) was used as medium for fungal growth. Normal saline was used to make spore suspension of fungal species (i.e., a loopful of particular fungal species was transferred to 3 ml of saline in order to obtain a suspension of corresponding species). Prepared Sabourauds agar media (20 ml) was poured in to each Petri dish. Excess of media was decanted and the plates were dried by placing in an incubator for 1h. Wells were made on these seeded agar plates using an agar punch and labeled. A 10µg/ml solution of the test compound in DMSO was then added in to each of these labeled wells. A control was also prepared in the same way using DMSO. The Petri dishes were then incubated at 37 ^{0}C for 3-4 days. The diameter of the inhibition zone was determined in comparison with the standard drug Amphotericin B. The results of antifungal studies are given in **Table 3.**

Table2. *Antibacterial activity data of compounds (4a-l)*

Compd.	Diameter of the inhibition zone (in mm)			
	S. aureus	***P. aeruginosa***	***K. pneumoniae***	***E. coli***
4a	18	17	24	19
4b	18	18	21	16
4c	19	17	23	20
4d	13	11	14	11
4e	16	17	21	17
4f	12	12	13	10
4g	15	11	20	13
4h	19	18	22	18
4i	10	10	17	12
4j	13	10	15	12
4k	16	13	10	12
4l	13	18	15	12
Ciprofloxacin	19	18	25	20

Diameter of the inhibition zone (in mm) at 10 µg/ml concentratio.

Table3. *Antifungal activity data of compounds (4a-l)*

Compound	Diameter of the inhibition zone (in mm)			
	A.fumigatus	*A. flavus*	*C. albicans*	*T. mentagraphytes*
4a	**20**	**18**	**20**	**19**
4b	17	15	16	11
4c	15	10	13	19
4d	13	10	17	12
4e	**22**	**15**	**17**	**20**
4f	19	18	14	17
4g	15	11	14	13
4h	**22**	**17**	**19**	**19**
4i	16	12	13	11
4j	15	17	12	15
4k	12	15	12	13
4l	14	14	12	11
Amphotericin-B	22	18	20	20

Diameter of the inhibition zone (in mm) at 10 µg/ml concentration

4. RESULTS AND DISCUSSION

The investigation of the antibacterial screening studies revealed that all the tested compounds **(4a-l)** showed moderate to good inhibition in respective solvents used for testing. The compounds **4a, 4b, 4c, 4e, 4h,** showed comparatively good activity against all the bacterial strains. The good activity can be attributed to the presence of pharmacologically active groups 4-methyl, 4-methoxy, 4-chloro, 4-phenyl, 4-methylthio, 4-fluoro, 2,3,4-trichloro which are directly attached to the phenyl ring of the pyrazoline and isoxazole ring system. The compounds **4a, 4e, 4h,** showed comparatively good activity against all the tested fungal strains. The groups 4-methyl, phenyl, 4-methylthio, which are directly attached to the phenyl ring of the pyrazoline and isoxazole ring were responsible for the good antifungal activity.

5. CONCLUSION

The research study reports the successful synthesis and antimicrobial activity of new Synthesis and evaluation of antimicrobial activities of some new prazoline derivatives carrying 4-methylthio moiety. The antimicrobial activity results indicated that **4a, 4b, 4c, 4e, 4h,** showed good antibacterial activity and 4a, 4e, 4h showed good antifungal activity.

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