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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDY OF COPPER COMPLEX OF 1-ACETYL-5-(4-NITROPHENYL)-3-(2-THIENYL)-2-PYRAZOLINE

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ABSTRACT

1-Acetyl-5-(4-nitrophenyl)-3-(2-thienyl)-2-pyrazoline was synthesized by the Claisen-Schmidt condensation of 3-(4-nitrophenyl)-1-(2-thienyl)-2-propene-1-one in presence of hydrazine hydrate in acetic acid and its copper complex have been synthesized and characterized on the basis of elemental analysis, magnetic susceptibility, molar conductance, molecular weight determination and spectral data like ^{1}H NMR, IR. These compounds were also screened for their antibacterial activity against Gram positive and Gram Negative bacteria.

Key words: 1-Acetyl-5-(4-nitrophenyl)-3-(2-thienyl)-2-pyrazoline, Copper complex, Antibacterial activity.

INTRODUCTION

Pyrazolines are well known important nitrogen containing five member heterocyclic compounds. They have only one endocyclic double bond and are basic in nature. A classical synthesis of these compounds involve the base-catalyzed aldol condensation reaction of aromatic ketones and aldehydes to give α, β-unsaturated ketones (chalcones), which undergo a subsequent cyclization reaction with hydrazines affording 2-pyrazolines[1]. They have found to possess antifungal[2-3], anticonvulsant[4], antidepressant[5], anti-inflammatory[6], antibacterial[7-8], anticancer[9], antioxidant[10], antiviral[11], antiamoebic[12] and antituberculosis[13-14] activities. Some of these compounds have also analgesic[15] activity and COX-2 inhibitor[16]. The prevalence of pyrazoline core in biological active molecules has stimulated the need for elegant and efficient ways to make these heterocyclic lead. Literature reports reveal that the activity of a ligand can be altered. Several folds by coordination with suitable metal ion[17], apparently due to the acceretion in lipophilicity of the metal chelates[18]. In the present study, we have investigated the interaction of Cu(II) with some newly synthesized pyrazolines. All the prepared compounds were screened for their antimicrobial activities.

EXPERIMENTAL

Materials and methods

All the reagents and solvents used were of laboratory grade. The synthesis of new products was monitored by TLC using (Ranbaxy) silica gel-G plates for TLC. IR spectra are recorded on Shimadzu

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FTIR 8400 (4000-400 cm^{-1}). ^1H NMR spectra are recorded on Bruker Avance III NMR 400 MHz spectrometer using TMS as internal standard. Conductance values were determined in dry DMSO at 10^{-3} M concentration on a digital conductivity meter NDC 732. C, H and N analysis were performed on an automatic elemental analyzer model Vario EL III. Magnetic susceptibility were measured on a sherwood magnetic susceptibility balance and copper was estimated by the atomic absorption spectrophotometer (Element AS, Model AAS 4141).

Synthesis of ligand

Synthesis of 1-acetyl-5-(4-nitrophenyl)-2-pyrazoline (HL_1)

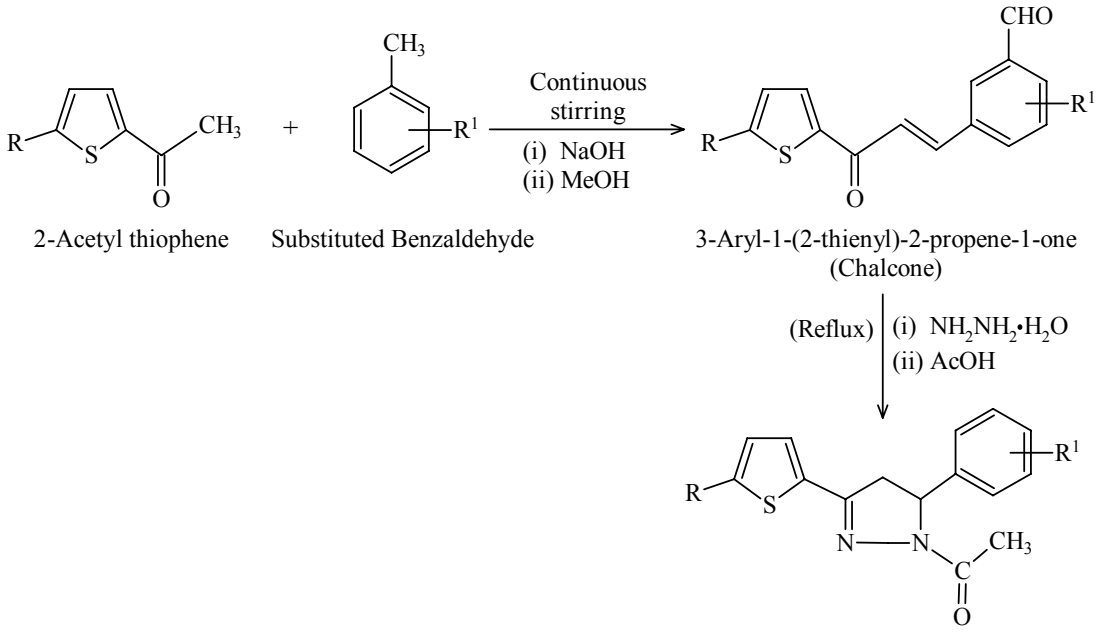
The ligand was synthesized in two steps:

Step-I: Synthesis of 3-(4-nitrophenyl)-1-(2-thienyl)-2-propene-1-one (chalcone)

Acetylthiophene (0.02 mol) was dissolved in 5 percent methanolic sodium hydroxide (30 mL) with constant stirring and 4-nitrobenzaldehyde (0.02 mol) was added dropwise into it at 0-5ºC with continuous stirring for 8 hr. The stirrer was removed and the reaction mixture was kept over night. The reaction mixture was poured on ice cold distilled water, neutralized with dilute sulphuric acid and filtered, washed with cold distilled water, dried and the resulting chalcone was purified by recrystallization from methanol.

Step-II: Synthesis of 1-acetyl-5-(4-nitrophenyl)-3-(2-thienyl)-2-pyrazoline (HL_1)

A solution of 3-(4-nitrophenyl)-1-(2-thienyl)-2-propene-1-one (0.01 mol) in acetic acid (35 mL) was refluxed with hydrazine hydrate (2.5 mL, excess) for 5-15 hrs. The progress of reaction was monitored by TLC. The reaction mixture was cooled overnight and poured onto ice-water. The separated solids was filtered, washed with distilled water, dried under vacuum and re-crystallized from methanol.



Ligand	R	R^1
HL_1	H	4-NO_2

Scheme 1: Synthesis of ligand (HL_1)

Ligand (HL_1) Yield: 70%, m.w. 315.2 g/mole; ^1H NMR (400 MH_Z, DMSO-D_6) δ: 7.073-8.256 (7H, m, Ar- H & thienyl H), 5.632-5.674 (1H, dd, C_5-H), 3.807-3.881 (1H, dd, C_4-Hcis), 3.111-3.168 (1H, dd, C_4-H trans), 2.408 (3H, s, $COCH_3$); IR (KBr) υ_{max} cm^{-1}: 1666 (C=O), 1512 (C=N), 1452, 1406 (C=C), 1342 (C-N), 835 (N-N), 707 (C-S): Anal. Calcd for $C_{15}H_{13}N_3O_3S$, C, 57.1; H, 4.1; N, 13.3%. Found C, 57.0; H, 4.2; N, 13.2%.

Synthesis of copper complex of 1-acetyl-5-(4-nitrophenyl)-3-(2-thienyl)-2-pyrazoline

A mixture of metal salt, copper chloride and ligand (1:2) in ethanolic medium was refluxed for 1-4 hrs. Metal Salt (0.002 mol) was dissolved in minimum amount of water and added slowly with continuous stirring of ethanolic ligand (0.004 mol) solution. The pH of the solution was maintained around 7 by adding 1% alcoholic ammonia solution. The resulting mixture was refluxed on water bath 4 hrs. A coloured product appeared on standing and cooling the above solution. The resulting precipitates was filtered off, washed several times with aqueous ethanol and dried under reduced pressure at 50-60ºC.

Copper complex: Yield: 64%, m.w. 764.91 g/mole; μ_{eff} (B.M.) 2.10; IR(KBr)υ_{max} cm^{-1}: 1616 (C=O), 1480 (C=N), 1344 (C-N), 849 (N-N), 705 (C-S), 500 (M=O), 441 (M-N), 285 (M-Cl); Anal. calcd for $C_{30}H_{26}Cl_2CuN_6O_6S_2$, C, 47.0; H, 3.4; N, 10.9; Cu, 8.3% Found C, 47.2; H, 3.5; N, 10.8; Cu 8.1%.

Antibacterial activity

Antibacterial activity of the ligand and metal complex was evaluated at Department of Microbiology, M. L. Sukhadia University, Udaipur (Raj.). The synthesized compounds were screened for antibacterial activity by Agar Disc Diffusion method[19] against Gram-positive bacteria *Micrococcus luteus*, *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli*. Nutrient agar (Microgen, India) was used for bacteria culture. The culture strains of bacteria were maintained on nutrient agar slant at 37 ± 0.5ºC for 24 hrs. The known compounds Amoxicillin was used as standard drug for antibacterial comparision study. The compounds were tested at a concentration at 500 µg/mL in DMSO. The diameter of zone of inhibition was measured in mm. DMSO was used as a control. Around 30 mL of sterile nutrient agar media for bacteria was poured into sterile petri dishes and allowed to solidify. The media was seeded with the organism by spread palate method using sterile L-roads and loops. Holes of 6 mm. diameter were punched carefully using a sterile cork borer and these were completely filled with the test solutions. The bacterial petri plates were kept in incubator at 37ºC for 24 hrs and then the zones of inhibition were measured.

Table 1: Antibacterial activity of ligand and its metal complex at 500 µg/mL (ppm)

Compounds	Zone of inhibition (in mm.)		
	Gram +ve		Gram –ve
	Micrococcus luteus	*Staphylococcus aureus*	*Escherichia coli*
$[Cu(HL_1)_2Cl_2]$	21	20	18
HL_1	17	16	15
Standard drug (Amoxicillin)	22	20	21

RESULTS AND DISCUSSION

In the present work, Claisen-Schmidt condensation of substituted aromatic ketone with aldehyde in alkaline methanol yielded 1, 3-diaryl-2-propen-1-one. The required ligand HL_1 was obtained by the reaction

of 1, 3-diaryl-2-propen-1-one with hydrazine hydrate in acetic acid. The copper complex was synthesized by reacting the respective ligand and metal ion solution in 2:1 stoichiometric ratio in alkaline medium. The synthesized ligand and complex were characterized by elemental analysis and spectral measurements. All the compounds are coloured solid, non-hygroscopic at room temperature. The ^{1}H NMR spectra of the ligand showed a multiple in the region 7.073-8.256 ppm assigned to the aromatic protons of phenyl and thienyl moieties. The acetyl protons appeared in the regions 2.408 ppm as singlets. The Cis C_4-H was absorbed at downfield 3.807-3.881 ppm as compared to its trans analogue 3.111-3.168 ppm. A double doublet in the region 5.632-5.674 ppm was assigned to C_5-H. The integral proton ratio of various groups in the spectrum of each ligand was tenable with the proposed structure.

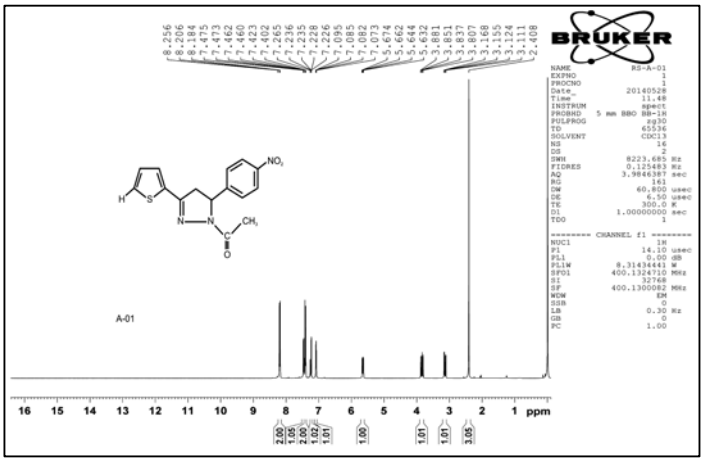


Fig. 1: ^{1}H NMR spectra of ligand [HL_1]

IR spectra of these ligand gave characteristic absorption frequencies in the region 1666 cm^{-1} and 1512 cm^{-1} assigned to υ (C=O) and υ (C=N) vibration, respectively. The stretching vibration for aromatic (C=C) appeared in the region 1452, 1406 cm^{-1} and υ (C-N) vibrations were observed in the regions 1342 cm^{-1}. A strong band in the region 835 cm^{-1} was attributed to υ (N-N). The thienyl C-S stretching vibrations were observed in the region 707 cm^{-1}.

Copper (II) complex suggested a bidentate behaviour of the ligand, which were found to coordinate through pyridyl nitrogen and carbonyl oxygen. The coordination through pyridyl nitrogen and carbonyl oxygen was indicated by negative spectral shift of υ (C=N) vibration from 1512 to 1480 cm^{-1} and υ (C=O) vibration from 1666 to 1616 cm^{-1}. The participation of nitrogen was further confirmed by shifting of υ (N-N) frequency to a higher wave number from 835 to 849 cm^{-1}. The bands at 707 cm^{-1} ascribed to thiophene ring stretching vibration in the spectra of ligand remained almost unchanged after complexation 705 cm^{-1}, clearly indicating the non-participation of sulphur or thienyl group in coordination. The non-ligand bands observed in the region 500, 441 and 285 cm^{-1} were assigned to υ (M-O), υ (M-N) and υ (M-Cl) modes, respectively.

The copper complex of HL_1, showed maximum activity against *M. luetus* as comparison with the ligand HL_1, which showed moderate activity. The copper complex displayed the highest antibacterial activity against *M. luteus* and *S. aureus* under study, this was because of increasing of lipophilic layer of these complex and the chelation process dominantly effects the biological behaviour of the complex that is potent against microbial strains. The biological activity of the ligand and its metal complex is less as compared to the standard drug, the complex are more active than ligand.

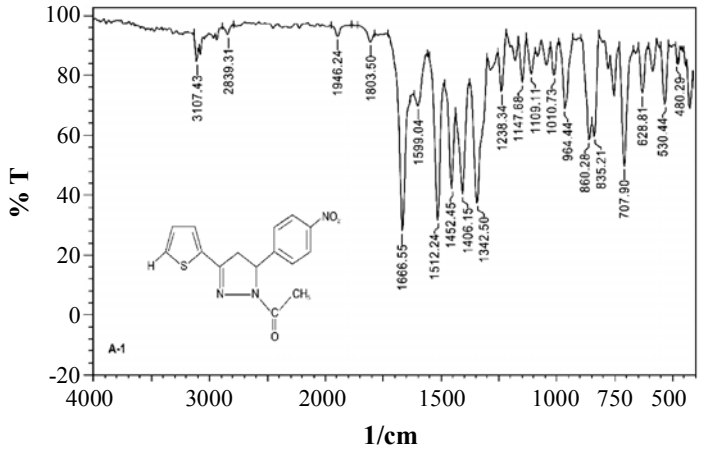


Fig. 2: IR spectra of ligand [HL_1]

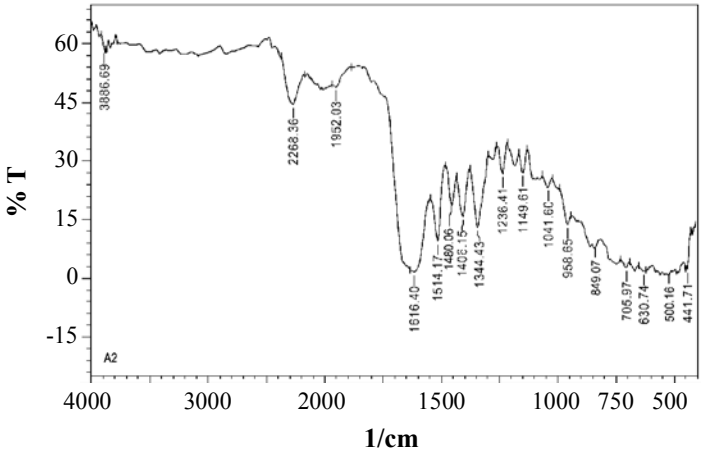


Fig. 3: IR spectra of [$Cu(HL_1)_2Cl_2$] complex

CONCLUSION

Present research work involves synthesis of novel pyrazoline derivative and their copper complex to explore their antibacterial activity. Copper complex exhibited highest antibacterial activity against *M. luteus*, *S. aureus* and *E. coli*. The observed increase in antibacterial activities is attributed to the presence of 4-NO_2 in phenyl ring and thienyl moiety of synthesized compounds. Hence, it is concluded that there is ample scope for further study in developing these as good lead compounds for the treatment of bacterial strains.

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