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Synthesis and antimicrobial studies of 4-diazo-5-mercapto-3-methyl/ethyl-s-triazoles and their metal chelates

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ABSTRACT

4-diazo-5-mercapto-3- methyl/ethyl-s-triazoles (1 & 2) were coupled with active methylene groups viz: acetyl acetone, ethylcyanoacetate and malanodinitrile to get dinucleating ligands (L_1-L_6). These ligands were reacted with Ni(II) and Cu(II) chlorides. Dinucleating ligands and metal complexes were characterized by elemental analysis, IR,NMR,ESR and magnetic movement studies. On the basis of physico- chemical studies Ni(II) and Cu(II) complexes have been assigned octahedral structure. The synthesized compounds have been screened for their antimicrobial activity.

Key words: 4-diazo-5-mercapto-3- methyl/ethyl-s-triazoles, dinucleating ligands, dinuclear complexes, antimicrobial activity.

INTRODUCTION

1,2,4-triazoles are associated with diverse pharmacological activities[1-3].Previous experimental and theoretical studies[4]in the field of adrenergic drugs have indicated that at least in the case of these type of drugs, the "methylene amino oxy methyl moiety" (=C=N-O-CH_2-, MAOMM) can be considered as a "biostere"[5] of either aryls or other aromatic or heterocyclic groups. Novel emerging major chemical groups as antimicrobials are triazole and triazole derivatives.[6-8] Triazoles, in particular, substituted 1,2,4- triazoles and the open chain thiosemicarbazide counterparts of 1,2,4-triazoles are among the various heterocycles that have received the most attention during the last two decades as potential anti-microbial agents.[9-10] Furthermore, diazonium salts of heterocycles, coupled with 2,4-pentadione have been used as precursor in the synthesis of antimicrobial triazines.[11]

Additionally, the involvement of metal ions in various biochemical reactions of living process is a matter of intensive and extensive investigations. There is substantial interest in the therapeutic activities of the coordination compounds as drugs[12] .

Our earlier report[13] on dinucleating ligands along with their metal complexes also prompted us to study more triazole groups than thiazole and benziimidazole groups as earlier showed better results for all activities.

Microwave assisted reactions using dry media have attracted much interest because of the simplicity in operation, greater selectivity and rapid synthesis of variety of heterocyclic compounds.[14] Thus it was thought worthwhile to synthesize the titled compounds (**1** and **2**) (Scheme -1) using the green route and also by conventional method.[15] These substituted triazoles were further diazotized and coupled with active methylene groups viz. acetyl acetone ,ethylcyanoacetate and malanodinitrile to get dinucleating ligands(L_1-L_6.) These dinucleating ligands were further complexed with Ni(II) and Cu(II) chlorides. The isolated dinuclear complexes along with the ligands were characterized and subjected to antimicrobial activity.

EXPERIMENTAL

Reagents and general techniques

Melting points are uncorrected. IR spectra were recorded on Thermo Nicolet FT IR spectrophotometer at Andhra University, ^{1}H NMR spectra were taken on Perkin Elmer R-32, 90 MHz in DMSO-d_6 using TMS as internal reference at IICT, Hyderabad and ESR spectra were taken on Varian E 112 at room temperature and as well as liquid nitrogen temperature using DPPH as standard at IIT Chennai. Thiocarbohydrazide, acetyl acetone , ethylcyanoacetate and malanodinitrile were purchased from Across . All the solvents were of analytical grade and were distilled before use. Microwave irradiation was carried out using a commercial microwave oven. All the complexes were analyzed for the metal content by standard procedure. Elemental analyses were carried out at Micro Analytical Center at Andhra University .The H.P.L.C. was recorded using Shimadzu LC 6A with Shimpack silica gel column.

Synthesis of 4-amino-5-mercapto-3-methy/ethyl-s-triazole (1 & 2)

A mixture of thiocarbohydrazide (2.5g,5mmol) and glacial acetic acid or propionic acid (15ml each in separate reactions) was heated under reflux on an oil bath for 5hrs,following the reported method[15].This reaction was also done in domestic Microwave oven .The time taken for this was only 5 minutes. . The reaction mixture was cooled to room temperature and the excess solvent was distilled off under reduced pressure. The residual solid was crystallized from methanol giving shining yellow crystals.

Synthesis of dinucleating ligands

0.1mol substituted amino-triazoles (1&2) were dissolved in 5ml conc HCl and cooled below -5^0 to -10^{0}C, by the addition of cold $NaNO_2$ (1.5g in 10ml H_2O) solution, it was diazotized (Scheme-1). In the resulting solution of diazonium salt, a cold solution of acetyl acetone ethylcyanoacetate and malanodinitrile (each 0.1mol separately) containing sodium acetate (5 g) in water (10 ml) was added. The mixture was stirred for 3hrs at 20°C .The solid product formed was collected by filtration and crystallized from EtOH. The isolated ligands (L_1-L_6). were characterized by various spectral methods.

General Procedure for the preparation of metal complexes

In the ethanolic solution of dinucleating ligand (L_1-L_6;1mmol each) an ethanolic solution of respective metal salts (2 mmol each $NiCl_2.6H_2O$, 0.46g; and $CuCl_2.H_2O$) was added separately[16] drop-wise while stirring followed by the addition of 2-3 drops of Et_3N. The contents of the mixture were refluxed for 3-4 hrs .The crystalline substance thus obtained were filtered and washed successively with ethanol and ether and dried in vacuum.

The dinucleating ligands were characterized by spectral methods. The analytical data of the ligands and their metal complexes are given in Table 1.

Study of anti microbial assay

The dinucleating ligands of substituted triazoles and their metal complexes were examined for antimicrobial assay using the well diffusion method[17,18] .200ml of nutrient agar growth medium was dispensed into sterile conical flasks ,these were then inoculated with 20µl of cultures mixed gently and poured into sterile petridish . After setting a borer with 6mm diameter was properly sterilized by flaming and used to make four uniform wells in each petridish. The wells were loaded with 50 µl of different investigated compounds. The solvent DMSO, used for reconstituting solvent for diluting the compounds were similarly analyzed for control. The plates were incubated at 37^{0}C for 24hrs. The above procedure is adopted for fungal assays also and the medium is potato dextrose agar (instead of nutrient agar) and incubated at 27°C for 48 hrs. The zone of inhibition was measured with a Hi Anti Biotic Zone Scale in mm and the experiment was carried out in duplicate. The results are shown in Table 3 and 4.

RESULTS AND DISCUSSION

Recently organic transformation accelerated under solvent free microwave irradiation condition gained wide popularity due to many practical advantages associated with enhanced reaction rates, high yields, improve selectivity and environment friendly reaction condition in tune with green chemistry. Keeping these facts in consideration, we adopted Microwave (MW)

Table 1: Physical and Analytical data of Synthesized Compound

S. No.	Compd	Colour/ M.P.(°C)	Yield %	Calcd(Found) % C	H	N	S	M	Cl
1	$L_1 = C_8H_{11}N_5SO_2$	Light yellow/203	65	39.82 (39.83)	4.54 (4.56)	29.06 (29.04)	13.20 (13.27)	-	-
2	$L_2 = C_8H_{10}N_6SO_2$	Llight yellow/182	85	37.79 (37.72)	3.93 (3.96)	33.07 (33.12)	12.59 (12.62)	-	-
3	$L_3 = C_6H_5N_7S$	Light yellow>250	63	34.78 (34.83)	2.41 (2.46)	47.34 (47.38)	15.45 (15.37)	-	-
4	$L_4 = C_9H_{13}N_5SO_2$	Light yellow>250	60	42.35 (42.28)	5.09 (5.11)	27.45 (27.57)	12.54 (12.66)	-	-
5	$L_5 = C_9H_{12}N_6SO_2$	Light yellow>250	72	40.29 (40.20)	4.47 (4.52)	31.34 (31.55)	11.94 (11.82)	-	-
6	$L_6 = C_7H_7N_7S$	Light yellow>250	75	38.00 (38.15)	3.16 (3.28)	44.34 (44.52)	14.47 (14.53)	-	-
7	$[Ni_2(L_1)Cl_4(H_2O)_4]$	Leafy green/>250	69	16.77 (16.73)	3.31 (3.39)	12.22 (12.35)	5.59 (5.68)	20.51 (20.71)	24.80 (24.92)
8	$[Cu_2(L_1)Cl_4(H_2O)_4]$	Leafy green/>250	62	16.44 (16.49)	3.79 (3.26)	12.24 (12.01)	5.32 (5.49)	21.98 (21.83)	24.65 (24.39)
9	$[Ni_2(L_2)Cl_4(H_2O)_4]$	Leafy green/>250	70	16.37 (16.20)	3.06 (3.12)	14.32 (14.35)	5.45 (5.55)	20.03 (20.31)	24.21 (24.45)
10	$[Cu_2(L_2)Cl_4(H_2O)_4]$	Leafy green/>250	60	16.09 (16.14)	3.01 (3.09)	14.08 (14.11)	5.36 (5.43)	21.30 (21.43)	23.80 (23.95)
11	$[Ni_2(L_3)Cl_4(H_2O)_4]$	Leafy green/>250	72	13.37 (13.44)	5.49 (5.55)	18.20 (18.31)	5.94 (5.88)	21.80 (21.87)	26.37 (26.45)
12	$[Cu_2(L_3)Cl_4(H_2O)_4]$	Leafy green/>250	60	13.13 (13.22)	2.37 (2.47)	17.88 (17.91)	5.83 (5.91)	23.18 (23.29)	25.90 (25.84)
13	$[Ni_2(L_4)Cl_4(H_2O)_4]$	Leafy green/>250	75	18.41 (18.45)	3.58 (3.65)	11.93 (11.88)	5.45 (5.52)	20.02 (20.15)	24..21 (24.35)
14	$[Cu_2(L_4)Cl_4(H_2O)_4]$	Leafy green/>250	60	18.11 (18.17)	3.52 (3.59)	11.74 (11.68)	5.36 (5.41)	21.31 (21.55)	23.82 (23.75)
15	$[Ni_2(L_5)Cl_4(H_2O)_4]$	Leafy green/>250	78	18.01 (18.08)	3.93 (3.97)	14.01 (14.08)	5.33 (5.39)	19.58 (19.72)	23.68 (23.44)
16	$[Cu_2(L_5)Cl_4(H_2O)_4]$	Leafy green/>250	60	!7.73 (17.70)	3.28 (3.24)	13.79 (13.72)	5.25 (5.28)	20.86 (20.98)	23.37 (23.45)
17	$[Ni_2(L_6)Cl_4(H_2O)_4]$	Leafy green/>250	75	15.15 (15.35)	2.70 (2.75)	17.67 (17.70)	5.77 (5.81)	21.17 (21.37)	25.61 (25.71)
18	$[Cu_2(L_6)Cl_4(H_2O)_4]$	Leafy green/>250	60	14.94 (14.90)	2.66 (2.69)	17.43 (17.54)	5.69 (5.72)	22.60 (22.82)	25.26 (25.33)

synthesis, for the first time We synthesized substituted triazoles (1 & 2) by MW as well as conventional methods .We had noticed that the time taken for completion of reaction is very less only 5 min as compared to conventional method (5hrs). The isolated compound 1 & 2 from both the methods were compared identical TLC and melting points (203°C for1 & 182°C for 2) were observed. These substituted triazoles were further diazotized and coupled with active methylene groups viz. acetyl acetone, ethylcyanoacetate and malanodinitrile to get dinucleating ligands(L_1-L_6.) These dinucleating ligands were characterized and then condensed with Ni (II) and Cu (II) chlorides.

IR spectra

The comparative IR spectral study of the ligands and their metal complexes reveal the interesting coordination mode of the ligand during complex formation. The disappearance of peaks in the region ~ 3370 cm^{-1} indicated that the NH_2 group of substituted triazole is changed to νN=N in the formation of ligands[16] which was further supported

Table 2: Powder Spin-Hamiltonian parameters of Cu(II) complex at Room temperature and Liquid Nitrogen temperature

S.No	Complex		g∥	g⊥	$A_{\parallel}$
1	$[Cu_2(L_1)Cl_4(H_2O)_4]$	RT	2.241	2.023	165
		LNT	2.322	2.042	165
2	$[Cu_2(L_2)Cl_4(H_2O)_4]$	RT	2.302	2.033	160
		LNT	2.342	2.023	165
3	$[Cu_2(L_3)Cl_4(H_2O)_4]$	RT	2.231	2..012	160
		LNT	2.200	2.033	160
4	$[Cu_2(L_4)Cl_4(H_2O)_4]$	RT	2.240	2.023	165
		LNT	2.302	2.033	160
5	$[Cu_2(L_5)Cl_4(H_2O)_4]$	RT	2.215	2.022	165
		LNT	2.252	2.120	160
6	$[Cu_2(L_6)Cl_4(H_2O)_4]$	RT	2.232	2.003	160
		LNT	2.242	2.103	160

Table 3: Biological Activity of Synthesized compounds against Bacteria at concentration of 1mg/ml

Compound	BS	EC	PV	E coli	EF	SF	KP	ML
L_1	7	6	7	8	8	7	8	NA
L_2	8	NA	8	7	7	6	7	NA
L_3	10	NA	10	6	9	6	8	7
L_4	8	7	6	8	8	8	5	8
L_5	9	NA	NA	9	6	5	7	6
L_6	11	7	NA	9	7	8	7	6
L_1-Ni	12	10	9	10	11	10	9	10
L_1-Cu	14	12	11	14	14	12	12	13
L_2-Ni	11	10	8	8	11	10	11	9
L_2-Cu	15	13	12	11	16	14	13	13
L_3–Ni	11	NA	9	10	9	8	NA	NA
L_3–Cu	14	10	12	11	11	10	9	10
L_4-Ni	10	NA	11	9	NA	NA	NA	NA
L_4–Cu	13	11	13	12	10	NA	8	9
L_5–Ni	9	9	11	9	NA	11	9	10
L_5–Cu	17	11	14	14	14	14	17	12
L_6-Ni	11	7	8	8	9	9	10	9
L_6-Cu	15	10	16	17	15	16	17	13

BS-*Bacillus subtilis,* EC-*Ervinia carotovora*, PV-*Pseudomonas vulgaris*, E.coli-*Escherichia coli*, EF-*Enterococcus faecalis*, SF-*Streptococcus faecalis*,KP-*Klebsilia pneuomonia*,ML-*Micrococcus luteus*, NA-Not active

Table 4: Biological activity of synthesized compounds against fungi at conc. of 1mg/ml

Compound	AF	PE	LT	RS
L_1	12	18	6	4
L_2	11	17	7	NA
L_3	12	15	8	NA
L_4	10	14	7	6
L_5	12	16	10	NA
L_6	12	16	15	NA
L_1-Ni	13	12	16	11
L_1-Cu	16	17	19	14
L_2-Ni	9	18	15	11
L_2-Cu	12	22	21	15
L_3–Ni	12	16	18	NA
L_3–Cu	15	19	19	13
L_4-Ni	13	14	17	11
L_4–Cu	18	21	22	14
L_5–Ni	12	17	16	11
L_5–Cu	19	23	23	17
L_6-Ni	11	16	18	11
L_6-Cu	20	22	23	18

AF-*Aspergillus flavus*, PE-*Pencillian expasom,*
LT-*Lasiodiplodia theobroma*,RS-*Rhizoctonia solani*
NA-Not active

by comparing NMR spectra of substituted triazole and dinucleatig ligands The peaks 1708(L_1), 1716(L_2),1705(L_4),1712 (L_5) and ~1421-1430(L_1 - L_6) respectively were assigned to νC=O and νN=N, which were shifted to lower frequencies[19] in the IR spectra of all metal complexes. The additional peak at 2222(L_2),2232 (L_3),2228(L_5) and2237 (L_6) was attributed to -CN[13]. The metal complexes showed new bands in the regions 480-450 cm^{-1} , which are due to the formation of M-N and M-O bonds[20] respectively.

^{1}H NMR Spectra

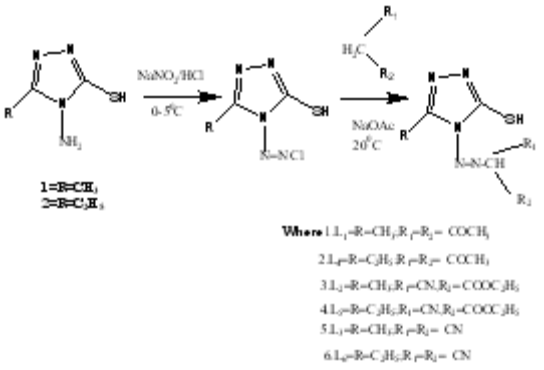
The ^{1}H NMR spectra of ligands (L_1 - L_6) were recorded in DMSO-d_6 . The disappearance of NH_2 peak and presence CH_3 at .~2.1 - 2.3 L_1, L_2 ,L_4 and L_5 indicated that substituted triazoles were diazotized and coupled[16,19] with acetyl acetone and ethycynoacetate. Whereas absence of NH_2 peak in the spectra of L_3 and L_6 was also supported the formation of N=N which was further condensed with malanodinitrile.The integration and position of other peaks were well fitted for other expected protons. Poor solubility of metal complexes restricted us to record NMR spectra.

ESR Spectra

The ESR spectra of Cu (II) complex of ligands were recorded at liquid nitrogen temperature(LNT) as well as room temperature (RT) .The spin Hamiltonian parameters, calculated from the spectra and reported in Table 2.The powder state spectra of the complexes at RT and LNT showed four equally spaced lines as expected for Cu(II) ion showing $g_{Q\%} > g_{4\%}$, however it is more clear in LNT spectra. The values are supportive of octahedral geometry around metal[21] . Basic spectral characteristics at both temperatures are the same with slightly better resolution[22] at LNT. The half field signal was not observed in any spectra is indicated that there is no Cu-Cu interaction between the complexes hence supportive of dinuclear complexes .

Anti microbial studies

Literature survey[1-4] and our earlier report[13] showed that substituted triazoles are having antimicrobial activity .In accordance with this synthesized compounds and their metal complexes were examined for antimicrobial assay against 8 bacteria [*BS-Bacillus subtilis, EC-Ervinia carotovora, PV-Pseudomonas vulgaris, E coli-Escherichia coli, EF-Enterococcus faecalis, SF-Streptococcus faecalis, KP-Klebsilia pneuomonia, ML-Micrococcus luteus*] and 4 fungi [AF-*Aspergillus flavus*, PE-*Pencillian expasom*, LT-Lasiodiplodia theobroma, RS-*Rhizoctonia solani*] given in Table 3and 4.



Scheme 1

It was observed that metal complexes were more active in both the cases Bacteria and Fungi. Moreover Cu complexes showed more activity against all bacteria. This can be correlated that water kept in copper vessels are good for drinking whereas Ni complexes were found more active against all fungi . Futher studies are under process.

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