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Antibacterial Activity Studies on Some Bioactive Mannich Bases and Their Metal Ion Complexes

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In this study, bioactive Mannich base 5-pyrrolidinomethyl uracil (PMU), dimethylaminomethyl uracil (DMAMU) and (5-diethylamino-methyl)-2-thio uracil (DEAMTU) and its metal ion complexes are synthesized by applying Mannich reaction using uracil/thiouracil as the active substrate secondary amine and formaldehyde. The structure of the complexes and ligands were characterized using spectral, elemental analysis and magnetic measurements. The Mannich bases were screened for their antibacterial activity against *S. pyogenes* and *K. pneumoniae*. The results showed that the metal chelates of the bioactive ligands have higher antibacterial activity than the free ligands.

Key Words: 5-Pyrrolidinomethyl uracil, Dimethylaminomethyl uracil, 5-Diethylaminomethyl-2-thiouracil, Metal complexes.

INTRODUCTION

The discovery of the disease causing germs has initiated discovery of the tools for the disinfection of the microorganisms in and around the human environment. Hence the search for the substances with antimicrobial activities is an important area of research. Certain chemicals of synthetic and plant origin are toxic to the bacteria and fungi but not to the host animal, certain bacteria and fungi develop drug-resistance on prolonged application of the drugs, making even a valuable drug in effective.

Metal complexes of Mannich bases have been studied extensively in recent years due to the selectivity and sensitivity of the ligands towards various metal ions[1-5]. The Mannich reaction is a three-component condensation reaction consisting of active hydrogen containing compound, formaldehyde and a secondary amine[6].

During the last two decades, there has been an increased interest in research activity in the field of coordination chemistry. The theoretical and experimental development of coordination chemistry has been continuously increasing as seen by a large number of research papers published in this area. It is also due to the development and refinement of ligand field and molecular orbital theories which could explain various experimental observations satisfactorily[7-9].

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Metal ions are known to play many important roles in biological processes. Though the metal content in an adult man is only 2 % of the body weight yet without these, life cannot sustain. So role of metal ions and metal complexes in biological processes has become increasingly important. Thus, the boundry between biochemistry and molecular biology on one side and inorganic chemistry on the other side has generated a new science called bio-inorganic chemistry.

The substituted (especially at the position) uracils and thiouracils are known to play a vital role in many metabolic processes most of which are also mediated by metal ions. The thiouracils have the potential to bind to the metal ion through sulfur, nitrogen, oxygen or a combination of these bindings are likely to leave some potential donor atoms free and these free donor atoms enhance the biological activity. The principal advantage of the Mannich reaction is that it enables two different molecules to be bonded together in one step. Hence this versality of the Mannich reaction along with the remarkable possibilities of exploiting the reactivity of Mannich bases in producing further derivatives makes it possible to readily attain the most varied chemical structures in conformity with the practical requirements and applications needed in industry.

Chemical substances which take part in cellular metabolic reactions are called metabolities and antimetabolities are a chemical agent, which by virtue of its close structural similarity to the metabolities blocks its action. The antimetabolities check the production of carcinogenic compounds produced by the abnormal metabolism of some organic compounds. Antimetablolite acts either by preventing the combination of the metabolite with its specific enzyme or it may combine itself with the specific enzyme, transforming the compound to an inactive one.

Substituted purines and pyrimidines constitute the backbone bases of RNA and DNA. Any disease which originates from gene repair will have vital implication of the daughter DNA and can be cured by meticulous incorporation of an antimetabolite to rectify the fault. Uracil 2,4-pyrimdinedione a nucleic acid base, is a building block of RNA. Any structural modification of uracil on the 5th position can generate an antimetabolite of thymine (5-methyl uracil) which is a building block of DNA.

5-Substituted block of pyrimidines have been suggested as possible intermediates in the transformation of thymine into RNA pyrimidines[10]. Essentially the drug gets into the cell and binds to specific gene implicated in an ailment. The bad gene in turn gets inactivated and stops producing the toxic protein permanently. This one time cure is more potent and cell likely to induce toxic side effects.

Several 5-substituted uracil and nitrogen mustards of uracil have been synthesized and tested for their *in vitro* and *in vivo* antitumour activity[11]. Accordingly thiopyridine also acts as potential inhibitor[12] and antimetabolite[13]. It has shown pronounced antibacterial activity[14] and has been found to be a basic constituent of some t-RNA[15]. It also exhibits antitumour and antithyroid activity because it readily incorporated into nucleic acids[16].

A compound derived form the combination of the alkylating center with a hydrophilic carrier moiety may be tumour specific and thus clinically useful. Likewise while looking for a biologically active substrate among pyridazine derivatives and has been the subject of several theoretical investigations[17]. Maleic hydrazide was found to be a potent inhibitor of leukemia[18] and its derivations are also used as novel bioactive agents[19] and especially Mannich-N-bases were proved to be pharmacologically more active than maleic hydrazide[20,21]. Derivations of maleic hydrazide can act as purine or pyrimidine analogue forming base pair with uracil and thymine by nucleoside formation through O or with adenine through N.

2-Arylimino-4-thiazolidione derivatives have various pharmacological activities such as antibacterial[22,23], antifungal[24] and anticancer[25]. Mannich bases have antimicrobial activities[26-28] besides various other activities. Mannich bases of some 2,5-disubstituted-4-thiazolidinone also have antimicrobial activity[29].

Biologically active Mannich bases with hetero aromatic ring system have been synthesized employing Mannich reaction of isonicotinyl hydrazide with various sulphonamides secondary amines. The Mannich bases were screened for their antibacterial activity against various gram positive and gram negative bacteria and were analyzed statistically. The results showed that the compounds are quite active against pathogen under study and were non-toxic[30].

The primary aim of the work is to analyze the antibacterial activity of various derivatives of uracil and thiouracil *i.e.* 5-pyrrolidinomethyl uracil (PMU), dimethylaminomethyl uracil (DMAMU) and (5-diethylaminomethyl)-2-thiouracil (DEAMTU) and its metal complexes (Table-1) against bacterial strains such as *S. pyogenes* and *K. pneumoniae*.

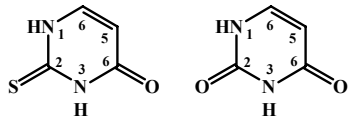
TABLE-1
MANNICH BASES AND ITS METAL COMPLEXES

Substrates	Mannich base	Metal complexes
Uracil + Formaldehyde + Pyrrolidine	PMU	$Co(NO_3)_2$·PMU $Ni(NO_3)_2$·PMU $Cu(NO_3)_2$·PMU $Zn(NO_3)_2$·PMU
Uracil + Formaldehyde + Dimethylamine	DMAMU	$Co(NO_3)_2$·DMAMU $Cd(NO_3)_2$·DMAMU $CdCl_2$·DMAMU $Zn(NO_3)_2$·DMAMU
Thiouracil + Formaldehyde + Diethylamine	DEAMTU	$Co(NCS)_2$·DEAMTU $(CoSO_4)_4$·DEAMTU·$4H_2O$ $CdBr_2$·DEAMTU $Zn(NO_3)_2$·DEAMTU

EXPERIMENTAL

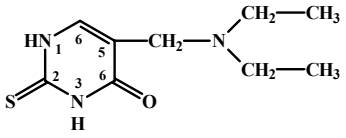
5-Pyrrolidinomethyl uracil (PMU), dimethylaminomethyl uracil (DMAMU) and (5-diethylaminomethyl)-2-thiouracil (DEAMTU) were synthesized by applying Mannich reaction using uracil/thiorucil as the active substrate, secondary amine and formaldehyde.

All samples used in this present work are well characterized and their structures are given below:



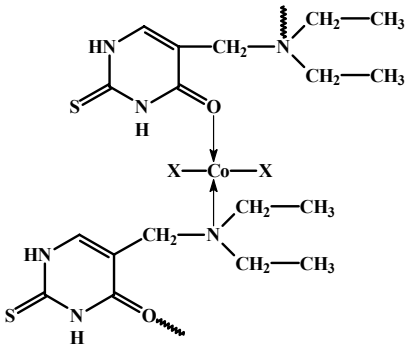
2-Thiouracil Uracil

Fig. 1



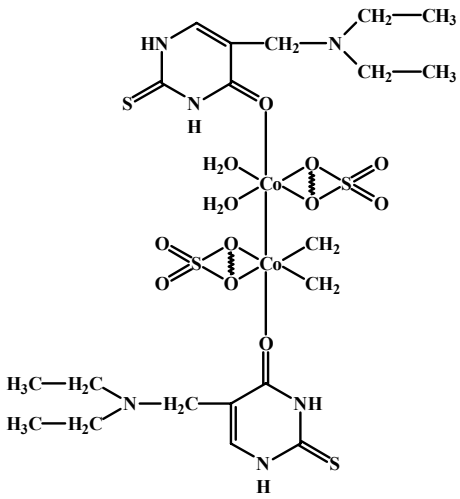
5-Diethylaminomethyl-2-thiouracil

Fig. 2



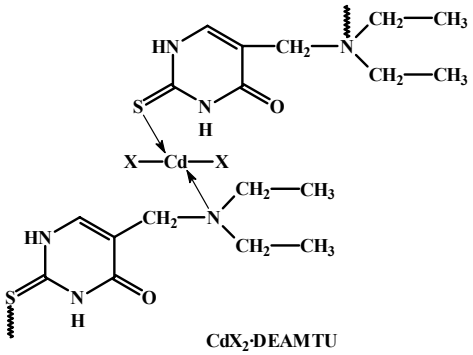
CoX_2·DEAMTU
X = Cl, Br, NO_3 and NCS

Fig. 3



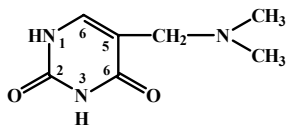
$(CoSO_4)_2$·$(DEAMTU)_2$·4 H_2O

Fig. 4



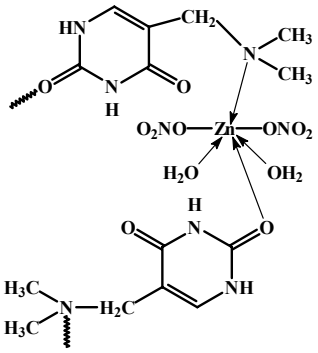
CdX_2·DEAMTU
X = Cl, Br and NO_3

Fig. 5



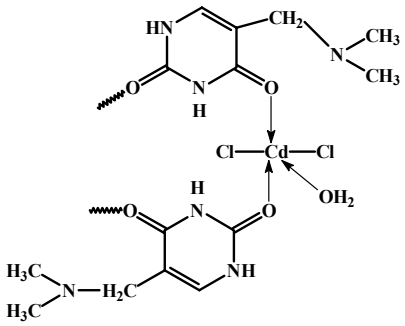
5-Dimethylaminomethyl uracil

Fig. 6



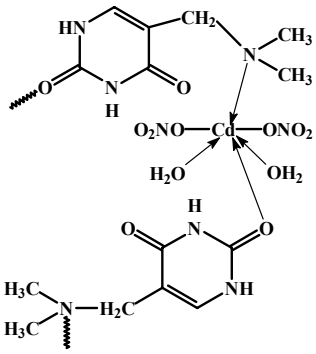
$Zn(NO_3)_2 \cdot DMAMU \cdot 2H_2O$

Fig. 7



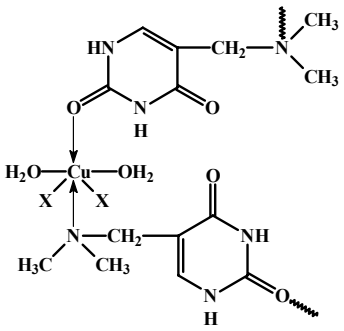
$CdCl_2 \cdot DMAMU \cdot 2H_2O$

Fig. 8



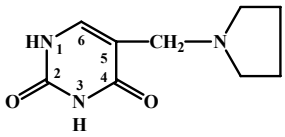
$Cd(NO_3)_2 \cdot DMAMU \cdot 2H_2O$

Fig. 9



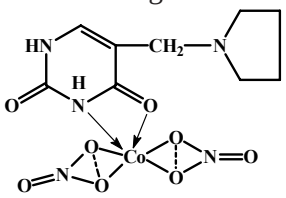
$CuX_2 \cdot DMAMU \cdot 2H_2O$
X = Cl and NO_3

Fig. 10



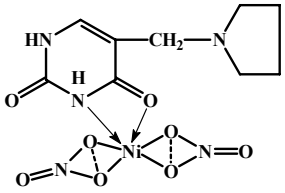
5-Pyrrolidinomethyl uracil

Fig. 11



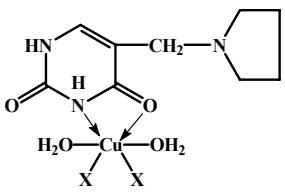
$Co(NO_3)_2 \cdot PMU$

Fig. 12



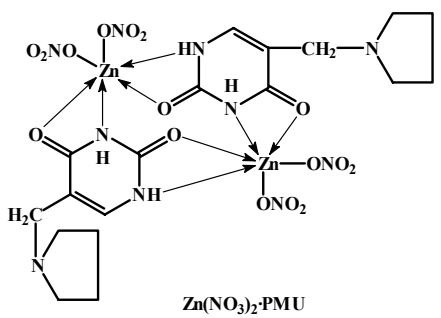
$Ni(NO_3)_2 \cdot PMU$

Fig. 13



$CuX_2 \cdot PMU \cdot 2H_2O$
X = NO_3

Fig. 14



$Zn(NO_3)_2$·PMU

Fig. 15

The antimicrobial activity of a drug can be tested by a wide range of techniques. Essentially a concentration gradient of the drug/test compound is produced in a nutrient medium and the growth or inhibition of growth of the organism taking place when the medium is seeded with test organism and incubated is observed. Many factors such as the kind of microorganism, the physiological state of the organism, the temperature, the medium or substrate carrying the organisms are important. The environmental segments must be considered in the application of any chemical agent to inhibit or destroy microbial populations.

Test compounds: Keeping the above facts in view, synthesized a derivative of uracil and thiouracil by selectively blocking one of the nitrogens and oxygen sits by substitution with carrier moieties like amines and enolization, respectively. Before testing, the test compound were dissolved in either DMF or DMSO.

The cultured bacterial strains *i.e.*, *Streptococcus pyogenes* and *Klebsiella pneumonia* were collected from Department of Eco-Biotechnology, Bharathidasan University, Trichy.

In the present study, the Agar diffusion technique[31] and serial tube dilution technique[32] were employed for antimicrobial assay.

Nutrient broth was prepared by dissolved peptone (1 g), meat extract (0.5 g) and sodium chloride (0.5 g) in distilled water (10 mL). The resulting solution was maintained at a pH 7.2-7.4. Nutrient agar was used for maintaining pure bacterial culture and to lawn the bacteria for detecting antibacterial activity. It was prepared by dissolving peptone (0.5 g) meat extract (0.2 g), sodium chloride (0.5 g) and agar (2.5 g) in distilled water (100 mL). The pH of the medium was maintained at 7.2-7.4. The bacteria was inoculated in the nutrient broth (inoculation medium) and kept in an incubator at 37 ºC for 24 h.

The bacterial subculture was taken in the seed layer medium for lawning. It consists of peptone (1 g), yeast extract (0.8 g), glucose (0.2 g), sodium chloride (0.6 g) and agar (0.2 g). These were dissolved in distilled water (100 mL) and sterilized.

A stock solution of the complex was prepared by dissolving 5 mg of the compound 5 mL of DMF so that the drug concentration is 500 µg/mL.

Agar diffusion method: The hot nutrient agar solution (20 mL) was poured into sterilized Petri dishes and allowed to attain room temperature. The seed layer medium was melted and cooled to about 45 ºC with gentle shaking. The previously grown subculture was added to the seed layer medium aseptically and mixed well. It was immediately lawned into the Petri dishes and allowed to attain room temperature. Then wells were made (10 mm diameter) with a sterile cork borer. To these wells, three different concentrations (0.20, 0.40, 0.60 mL) of the drug solution were added and the plates were allowed to cool for an hour to facilitate the diffusion. The plates were incubated at 37 ºC for 48 h. At the end of inoculation period, the zones of inhibition around the wells were measured. Tetracycline was used as reference standard.

Serial tube dilution method: In the serial tube dilution method, a nutrient broth medium containing 1 % (w/v) peptone, 0.5 % (w/v) yeast extract and 0.5 % (w/v) sodium chloride was prepared in distilled water and sterilized by autoclaving for about 0.5 h. A standard volume (20 mL) of the nutrient broth medium that would support the growth of the test organism was added to several labelled sterile identical assay tubes. A solution of each test compound was prepared in DMF and a series of dilutions were prepared using sterile pipettes. The concentrations tested were 150, 200 and 250 µg/mL of the compound under investigation, a wide spectrum antibiotic, the respective metal salt and the blank (DMF). A control tube containing no test compound was also included. Same quantity of the test organism from the broth culture was also added. All these operations were carefully carried out under aseptic conditions. The assay tubes were incubated at 37 ± 1ºC. The resultant turbidities at 24 and 48 h were measured in a Nephelo-turbidity meter.

The minimum inhibitory concentration of a test compound is the least concentration showing no visible turbidity. However, the percentage of bacterial growth inhibition produced by a particular concentration of the test compound was calculated form the measure of the turbidity of the control and turbidity of the particular treatment, provided, the impact of the solvent and metal on the growth of the organism are negligible. The relationship used is:

$$\% \text{ of Inhibition} = \frac{T_c - T_t}{T_c} \times 100$$

where, T_c = turbidity of the control, T_t = turbidity of the specific treatment or the test compound.

The inhibition of growth of the gram-positive organism (*S. pyogenes*) produced by various concentrations of the test compounds was compared under identical conditions with the inhibition of the growth of the same by tectracycline, which is a standard antibiotic active against gram-negative organisms (*K. pneumoniae*) produced by the test compounds was wide spectrum antibiotic. In all the compounds tested for antimicrobial assay, it is found that activity increases with increase in concentration of the test compound.

The antibacterial of all the compounds were also tested by the serial tube dilution technique. The percentage of inhibition produced by the different concentration of the compounds in this technique were almost same as the zone of inhibition produced in the Agar diffusion technique.

RESULTS AND DISCUSSION

The data obtained for the antibacterial activity of the ligands and their metal complexes against the bacterial above are presented in Tables 2-7.

TABLE-2
ANTIBACTERIAL ACTIVITY OF 5-PYRROLINOMETHYL URACIL (PMU) AND ITS METAL COMPLEXES (BY AGAR DIFFUSION TECHNIQUE)

Compound	Concentration (μg/mL)	Zone of inhibition (mm)	
		S. pyogenes	*K. penumoniae*
PMU	0.20	13	13
	0.40	14	15
	0.60	17	17
$Co(NO_3)_2$·PMU	0.20	23	19
	0.40	24	23
	0.60	26	26
$Cu(NO_3)_2$·PMU	0.20	20	15
	0.40	22	17
	0.60	24	18
$Ni(NO_3)_2$·PMU	0.20	16	18
	0.40	17	20
	0.60	19	23
$Zn(NO_3)_2$·PMU	0.20	16	17
	0.40	18	19
	0.60	20	21

Antibacterial activity

Antibacterial activity PMU and its metal ion complexes: The bioactive ligand PMU exhibits moderate activity. Among the PMU complexes tested, cobalt(II) nitrato complex is found to the most active against *S. pyogenes* and *K. pneumoniae* (Tables 2 and 5). The activity of the Co(II) complex may be due to its interaction with RNA as discussed earlier.

The order of activity of PMU complexes against *S. pyogenes* is:

$Co(NO_3)_2$·PMU > $Cu(NO_3)_2$·PMU > $Ni(NO_3)_2$·PMU > $Zn(NO_3)_2$·PMU > PMU

The order of activity of PMU complexes against *K. pneumoniae* is:

$Co(NO_3)_2$·PMU> $Ni(NO_3)_2$·PMU > $Zn(NO_3)_2$·PMU > $Cu(NO_3)_2$·PMU > PMU

Antibacterial activity of DMAMU complexes: The ligand DMAMU exhibits moderate activity. Among the DMAMU complexes, the activity of Co(II) nitrato complex has the highest and the least active is Zn(II) nitrato complexes against *S. pyogenes* and *K. pneumoniae* (Tables 3 and 6). This observation clearly indicates that the chelation increases the activity. The higher activity of Co(II) complex may be due to the fact that Co(II) is an essential micronutrient during transcription and transformation of nucleic acids.

TABLE-3
ANTIBACTERIAL ACTIVITY OF DIMETHYLAMINOMETHYL URACIL (DMAMU) AND ITS METAL COMPLEXES (BY AGAR DIFFUSION TECHNIQUE)

Compound	Concentration (μg/mL)	Zone of inhibition (mm)	
		S. pyogenes	*K. pneumoniae*
DMAMU	0.20	14	12
	0.40	16	13
	0.60	17	15
$Co(NO_3)_2$·DMAMU	0.20	18	19
	0.40	22	24
	0.60	26	27
$Cd(NO_3)_2$·DMAMU	0.20	20	21
	0.40	22	18
	0.60	25	20
$CdCl_2$·DMAMU	0.20	18	20
	0.40	19	23
	0.60	20	28
$Zn(NO_3)_2$·DMAMU	0.20	16	15
	0.40	18	16
	0.60	19	18

TABLE-4
ANTIBACTERIAL ACTIVITY OF (5-DIETHYLAMINOMETHYL)-2-THIOURACIL (DEAMTU) AND ITS METAL COMPLEXES (BY AGAR DIFFUSION TECHNIQUE)

Compound	Concentration (μg/mL)	Zone of inhibition (mm)	
		S. pyogenes	*K. penumoniae*
DEAMTU	0.20	14	15
	0.40	15	16
	0.60	16	17
$Co(NCS)_2$·DEAMTU	0.20	16	14
	0.40	18	17
	0.60	20	20
$CoSO_4$·DEAMTU	0.20	15	16
	0.40	17	18
	0.60	18	19
$CdCl_2$·DEAMTU	0.20	12	13
	0.40	13	15
	0.60	17	17
$Zn(NO_3)_2$·DEAMTU	0.20	12	13
	0.40	13	14
	0.60	15	16

The order of activity of DMAMU complexes against *S. pyogenes* is:
$Co(NO_3)_2$·DMAMU > Cd $NO_3)_2$·DMAMU > $CdCl_2$·DMAMU > $Zn(NO_3)_2$·DMAMU > DMAMU

The order of activity of DMAMU complexes against *K. pneumoniae* is:
$Co(NO_3)_2$·DMAMU > $CdCl_2$·DMAMU > $Cd(NO_3)_2$·DMAMU > $Zn(NO_3)_2$·DMAMU > DMAMU

TABLE-5
ANTIBACTERIAL ACTIVITY OF 5-PYRROLINOMETHYL URACIL (PMU) AND ITS METAL COMPLEXES (BY SERIAL TUBE DILUTION METHOD)

Compound	Concentration (µg/mL)	% Inhibition			
		S. pyogenes		*K. pneumoniae*	
		24 h	48 h	24 h	48 h
PMU	150	55	55	52	65
	200	62	62	56	70
	250	70	70	60	72
$Co(NO_3)_2$·PMU	150	65	82	68	80
	200	70	86	70	84
	250	76	88	73	85
$Ni(NO_3)_2$·PMU	150	63	70	65	72
	200	65	74	70	76
	250	68	78	72	80
$Cu(NO_3)_2$·PMU	150	58	72	58	66
	200	62	76	62	68
	250	64	78	64	72
$Zn(NO_3)_2$·PMU	150	60	68	54	70
	200	53	72	56	72
	250	65	76	60	78
Standard	150	72	86	75	84
	200	76	88	82	86
	250	80	90	84	92

TABLE-6
ANTIBACTERIAL ACTIVITY OF DIMETHYLAMINOMETHYL URACIL (DMAMU) AND ITS METAL COMPLEXES (BY SERIAL TUBE DILUTION METHOD)

Compound	Concentration (µg/mL)	% Inhibition			
		S. pyogenes		*K. pneumoniae*	
		24 h	48 h	24 h	48 h
DMAMU	150	48	65	53	55
	200	62	70	58	62
	250	65	74	66	70
$Co(NO_3)_2$·DMAMU	150	63	66	65	70
	200	65	72	70	73
	250	68	79	76	77
$Cd(NO_3)_2$·DMAMU	150	48	55	50	63
	200	52	62	53	65
	250	65	70	60	68
$CdCl_2$·DMAMU	150	46	52	43	52
	200	50	55	54	60
	250	53	60	58	68
$Zn(NO_3)_2$·DMAMU	150	42	52	43	55
	200	48	60	45	70
	250	50	65	52	78
Standard	150	65	68	65	74
	200	72	76	73	80
	250	78	82	79	86

TABLE-7
ANTIBACTERIAL ACTIVITY OF (5-DIETHYLAMINOMETHYL)-2-THIOURACIL (DEAMTU) AND ITS METAL COMPLEXES (BY SERIAL TUBE DILUTION METHOD)

Compound	Concentration (μg/mL)	Zone of inhibition (mm)			
		S. pyogenes		*K. pneumoniae*	
		24 h	48 h	24 h	48 h
DEAMTU	150	46	52	44	52
	200	50	60	48	64
	250	60	67	58	69
$Co(NCS)_2$·DEAMTU	150	58	64	56	66
	200	64	72	62	72
	250	72	84	70	86
$CoSO_4$·DEAMTU	150	54	64	52	62
	200	62	72	60	70
	250	70	80	72	78
$CdCl_2$·DEAMTU	150	45	62	47	58
	200	52	68	56	62
	250	68	74	64	74
$ZnCl_2$·DEAMTU	150	44	60	40	57
	200	50	58	54	63
	250	65	72	61	75
Standard	150	66	68	63	76
	200	74	76	69	81
	250	78	80	75	89

Antibacterial activity of DEAMTU complexes: Since most of the cobalt(II) complexes of DEAMTU are insoluable in DMF, only the Co(II) thiocyanate complex is taken for studying the antibacterial activity. Among the various complexes (Tables 4 and 7) screened the cobalt(II) complexes was found to be most active.

The order of activity of DEAMTU complexes against *S. pyogenes* is:

$Co(NCS)_2$·DEAMTU > $CoSO_4$·DEAMTU > $CdBr_2$·DEAMTU > DEAMTU > $Zn(NO_3)_2$·DEAMTU

The order of activity of DEAMTU complexes against *K. pneumoniae* is:

$Co(NCS)_2$·DEAMTU > $CoSO_4$·DEAMTU > $CdBr_2$·DEAMTU > DEAMTU > $Zn(NO_3)_2$·DEAMTU

Mannich bases of 5-non-subtituted-2-substituted-4-thiazolidinones where the tested compounds has shown significant antibacterial activity. From these values, it is interesting to note that the ligand is less potent bactericide than the metal complexes. There are reports that antimicrobial properties of some organic compounds are considerably enhanced by complexation with transition metal ion[33].

The results of the screening of PMU, DMAMU and DEAMTU and their metal ion complexes against *S. pyogenes* and *K. pneumoniae* by agar diffusion and serial tube dilution techniques were adopted to evaluate their activity. It has been observed that the increase in concentration increases the activity. It has also been found that the metal chelated of the bioactive ligand have higher antibacterial activity than the free ligands. Among the ligands pyrrolydinomethyl substituted pyrimidines show

higher activity than the other two ligands. The order of activity is found to be PMU > DMAMU > DEAMTU. In the case of metal complexes, Co(II) and Cu(II) complexes of these ligands were found to be more active in majority of the cases.

Metal chelates of sulphur containing ligands have received major attention of biochemists because of their versatile use as antimicrobial agents[34,35] although several antimicrobial agents containing sulphur were discovered more than 50 years ago, relatively little is known about their mode of action. It is realized that the ligand systems with -NNS, -NNO, -ONO>C=S and >C=O moieties posses fairly good antimicrobial activity[36,37].

Conclusion

The study was conducted with the objective of find out the efficacy of antimicrobial activities to PMU, DMAMU and DEAMTU and their metal ion complexes against *S. pyogenes* and *K. pneumoniae*.

The results of antimicrobial activity show that the metal complexes exhibit antimicrobial properties and it is important to note that they showed enhanced inhibitory activity compared to the parent ligand. It has also been proposed that concentration play a vital role in increasing the degree of inhibition; as the concentration increases, the activity increases.

The results obtained for antibacterial activity of Mannich bases and its metal complexes were reported in Tables 2-7. The antimicrobial assessment revealed that the prepared compound mostly only a moderate activity. Analysis of data revealed that among the tested compounds, the PMU has highest rates of antimicrobial activity compared to DMAMU and DEAMTU.

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