EVALUATION OF ANTIMICROBIAL ACTIVITIES OF NAPHTHOIC ACID AND ITS DERIVATIVES

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Abstract : ***A newly synthesized lanthanum complex like 1- naphthoic acid, 2-naphthoic acid, 1-hydroxy-2-naphthoic acid, 2-hydroxy-1-naphthoic acid, 3-hydroxy-2-naphthoic acid and 2-napthoxy acetic acid have been screened for antibacterial activity by MIC method and results were compared with the activity of the un complexed antibiotic against Escherichia coli and Staphylococcus aureus. The metal complexes were found to be more potent against one or more bacterial species than the uncomplexed Ciprofloxacin.***

Index Terms - Escherichia coli; Antibacterial activity; Naphthoic Acid

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I. INTRODUCTION

Hydrazones and their metal complexes have been found to be potential application in industry and biology (Orvig et al 1998). The antibacterial properties of lanthanide (III) complexes have attracted the interest of researchers (Gudasi et al 2007 & Mohanan 2008); their chemistry and pharmacological applications have been extensively investigated. These have emerged as important class of nitrogen and oxygen or sulfur ligands particularly for transition and inner transition metal ions in the last two decades. Schiff base lanthanide complexes have been widely studied because they have industrial, antifungal, antibacterial, anticancer and herbicidal applications (Sahebalzamania et al 2010).

Tetracycline complexes of lanthanide were tested in vitro to evaluate their activity against the bacterial strains Escherichia coli and Staphylococcus aureus (Mutallik et al 2011). The Schiff base ligand forms very stable complexes with the lanthanide metals La, Ce, Pr, Nd, Sm, Gd, Tb, Dy and Er, their structural, spectroscopic, biological properties have been reported (Ajitha et al 2010). The ligands behave in bidentate fashion coordinating through hydrazide >C=O and nitrogen of >C=N. A coordination number of ten is assigned to the complexes. The Antibacterial and antifungal studies indicate an activity of the ligands on complexation (Agarwal et al 2009).

The rare earth elements (lanthanides) have inhibitory activity against bacteria and that they are used as antiseptic medicines. They often are applied as complexes with inorganic ligands as well as with organic ones. The antibacterial action depends on the concentration of lanthanide ions. High concentration inhibits growth of bacteria whereas low concentration stimulates it (Brzyska et al 1999). The synthesis, characterization, fluorescent and antimicrobial properties of new Lanthanide(III) complexes derived from coumarin Schiff base have been reported (Venkatesh Mutalik et al 2011). Based on the observation the newly synthesized complexes were screened for their biological activities.

2. EXPERIMENTAL SECTION

2.1 Synthesis of $La(N_2H_4)_2(1/2\text{-}C_{10}H_7COO)_3].2H_2O$, $[La(N_2H_4)_2\{C_{10}H_6(x\text{-}O)(y\text{-}COO)\}_{1.5}].zH_2O$ (x = 1, 2 & 3; y = 1, 2) and $[La(N_2H_4)_2\{2\text{-}C_{10}H_7OCH_2(COO)\}_3].3H_2O$

The respective metal oxide (for e.g., La_2O_3, 0.325 g, 1 mmol) was dissolved in a minimum quantity of 1:1 HNO_3, evaporated to eliminate excess of acid, and dissolved in 20 mL of water. To a freshly prepared aqueous solution (60 mL) of the ligands containing naphthoic acid and hydroxy naphthoic acid (0.172 g, 1 mmol) and hydrazine hydrate (0.2 g, 4 mmol). The solution was heated over hot water bath at 70 °C, for about 15 minutes. Then to the clear solution at pH 6, was added to the metal solution and stirring the reaction mixture vigorously. A microcrystalline solid formed immediately. Then the complex was filtered, washed with water, alcohol and then with ether and dried in a desiccators over anhydrous $CaCl_2$.

2.2. Collection and Maintenance of Test Organisms

The organisms used were clinical isolates of gram positive bacteria Staphylococccus aureus and gram negative bacteria Escherichia coli (from KMCH Hospital, Coimbatore). They were collected in McCartney bottles containing nutrient agar slants.

2.3 Preparation of Inoculums

The inoculums for the experiment were prepared in fresh Nutrient broth from preserved slant culture. The inoculums were standardized by adjusting the turbidity of the culture to that of McFarland standards. The turbidity of the culture may be adjusted by the addition of sterile saline or broth (if excessive or by further incubation to get required turbidity (Leonard Jarrett et al).

2.4 Preparation of Sterile Swabs

Cotton wool swab on wooden applicator or plastics were prepared and sterilized by autoclaving or dry heat (only for wooden swabs) by packing the swabs in culture tubes, papers or tins etc.

2.5. Sterilization of Forceps

Sterilize forceps by dipping in alcohol and burning off the alcohol.

2.5 Procedure (Zone of inhibition)

The standardized inoculums is inoculated in the plates prepared earlier (aseptically) by dipping a sterile in the inoculums removing the excess of inoculums by passing by pressing and rotating the swab firmly against the side of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60 ºC after each application. Finally pass the swab round the edge of the agar surface. Leave the inoculums to dry at room temperature with the lid closed.

Each Petri dish is divided into 4 parts, in three parts samples disc such as 3-hydroxy -2-naphthoic acid, 2-hydroxy-1-naphthoic acid, 1-hydroxy-2-naphthoic acid, 1- naphthoic acid, 2-napthoic acid and 2-napthoxy acetic acid (100µg) disc (discs are soaked overnight in

sample solution) and one quadrant for Std Ciprofloxacin 10µg, are placed in each plate with the help of sterile forceps. Then Petri discs are placed in the refrigerator at 4 ºC or at room temperature for 1 hour for diffusion. Incubate at 37 ºC for 24 hours. Observe the zone of inhibition produced by different Antibiotics (Table 1). Measure it using a scale and record the average of two diameters of each zone of inhibition.

2.6. Minimum Inhibitory Concentration

2.6.1. Preparation of test drug

Serial 2-fold dilutions of the test antimicrobial agent were made in 1ml of Muller Hinton Broth. Series of 10-15 dilutions to final concentrations of 100-1.56µg/ml are prepared.

2.6.2. Preparation of inoculums

Overnight culture are grown at 37 ºC Kirby- Bauer procedure and diluted to Muller Hinton Broth. This overnight culture was diluted to 10^{-2}.

- The sterile tubes were labelled 1-8 and 8th tube was taken as control.
- 1ml of Muller Hinton Broth was transferred to all tubes except 6th and 7th.
- 0.5ml of broth was transferred to 6th & 7th tubes.
- 1ml of drug solution was added to 1st tube and mixed well.
- From the 1st tube transfer 1ml of solution to the 2nd tube and was repeated up to 6th tube.
- From the 6th tube 0.5ml of solution was taken and transferred to 7th tube.
- 0.01ml of culture was added to all the test tubes.
- All the tubes were incubated at 37 ºC for 18-24hrs.
- After incubation observe the turbidity or OD value by Spectrophotometric method.

Antibacterial activities of six systems of naphthoic and substituted naphthoic acid complexes are tested against two bacteria such as S. Aurease, Escherichia.Coli. Ciprofloxacin was used as standard drug. Naphthoic and substituted Naphthoic acid complexes have shown remarkable antibacterial activity, the results of antibacterial activity are presented in Table 1.

Table 1 Antimicrobial Activity of Synthesized Complexes

COMPLEXES	Zone of inhibition	
	Gram - Negative	Gram - Positive
	Escherichia coli	*Staphylococcus aureus*
$La(N_2H_4)_2(1\text{-}C_{10}H_7COO)_3].2H_2O$	12	18
$La(N_2H_4)_2(2\text{-}C_{10}H_7COO)_3].2H_2O$	11	19
$[La(N_2H_4)_2\{C_{10}H_6(1\text{-}O)(2\text{-}COO)\}_{1.5}].3H_2O$	18	16
$[La(N_2H_4)_2\{C_{10}H_6(2\text{-}O)(1\text{-}COO)\}_{1.5}]$	18	15
$[La(N_2H_4)_2\{C_{10}H_6(3\text{-}O)(2\text{-}COO)\}_{1.5}].H_2O$	19	12
$[La(N_2H_4)_2\{2\text{-}C_{10}H_7OCH_2(COO)\}_3].3H_2O$	10	09
SD*	21	20

SD* - Standard drug- Ciprofloxacin

The complexes were sensitive to E. coli and S. aureus organisms with inhibitory zones within 9-19 mm. Higher activity observed against the gram negative bacteria E.coli for $[La(N_2H_4)_2\{C_{10}H_6(3\text{-}O)(2\text{-}COO)\}_{1.5}].H_2O$ complex. For gram positive bacteria S. Aureus for $La(N_2H_4)_2(2\text{-}C_{10}H_7COO)_3].2H_2O$ complex.

Minimal Inhibitory Concentration (MIC) was further determined using the method of progressive dilution in liquid media containing 15.625 to 1000 µg/mL of the complex being tested. The results were shown in Table 2.

Table 2 Minimum inhibitory concentrations of bacterial strains of naphthoic and substituted naphthoic acid complexes by serial dilution method

No.	Complexes	Organisms	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml	15.625 µg/ml
1	$La(N_2H_4)_2(1\text{-}C_{10}H_7COO)_3].2H_2O$	*E.coli*	-	-	-	-	-	+	+
		S.aureus	-	-	-	-	-	+	+
2	$La(N_2H_4)_2(2\text{-}C_{10}H_7COO)_3].2H_2O$	*E.coli*	-	-	-	-	+	+	+
		S.aureus	-	-	-	-	-	+	+
3	$[La(N_2H_4)_2\{C_{10}H_6(1\text{-}O)(2\text{-}COO)\}_{1.5}].3H_2O$	*E.coli*	-	-	-	-	-	+	+
		S.aureus	-	-	-	-	-	+	+
4	$[La(N_2H_4)_2\{C_{10}H_6(2\text{-}O)(1\text{-}COO)\}_{1.5}]$	*E.coli*	-	-	-	-	-	+	+
		S.aureus	-	-	-	-	-	+	+
5	$[La(N_2H_4)_2\{C_{10}H_6(3\text{-}O)(2\text{-}COO)\}_{1.5}].H_2O$	*E.coli*	-	-	-	-	-	+	+
		S.aureus	-	-	-	-	+	+	+

6	$[La(N_2H_4)_2\{2\text{-}C_{10}H_7OCH_2(COO)\}_3].3H_2O$	*E.coli*	-	-	-	+	+	+	+
		S.aureus	-	-	-	+	+	+	+

Among all the complexes $[La(N_2H_4)_2\{C_{10}H_6(1\text{-}O)(2\text{-}COO)\}_{1.5}].3H_2O$, $[La(N_2H_4)_2\{C_{10}H_6(2\text{-}O)(1\text{-}COO)\}_{1.5}]$ & $[La(N_2H_4)_2\{C_{10}H_6(3\text{-}O)(2\text{-}COO)\}_{1.5}].H_2O$ complexes showed very good activity against E.coli organisms (MIC = 62.5 g/mL), but $[La(N_2H_4)_2\{2\text{-}C_{10}H_7OCH_2(COO)\}_3].3H_2O$ complex was least active.

However, the synthesized complexes showed relatively higher or lower active than the standard drug Ciprofloxacin. The activity of any compound is a complex combination of steric, electronic and pharmacokinetic factors. A possible explanation for the toxicity of the complexes is postulated in the light of chelation theory. It is suggested that the chelation considerably reduces the charge of the metal ion mainly because of partial sharing of its positive charge with the donor groups and possible π- electron delocalization over the whole chelate ring.

This increases the lipophilic character of the metal chelate which favours its permeation through lipoid layers of cell membranes. Furthermore, the mode of action of the compounds may involve the formation of a hydrogen bond through the -N=C group of the chelate or the ligand with the active centres of the cell constituents resulting in interference with the normal cell process. The higher bacteria toxicity experienced by the compounds may be ascribed to the fact that the ligand and metal ions are more susceptible towards the bacterial cells. Table 3 shows the Minimum inhibitory concentration of synthesized complexes.

Table 3 Minimum inhibitory concentration of lanthanide complexes of naphthoic and substituted naphthoic acids and hydrazine

S.NO	Complexes	Organisms (MIC)	
		E. coli	S. aureus
1	$La(N_2H_4)_2(1\text{-}C_{10}H_7COO)_3].2H_2O$	62.5µg/ml	62.5µg/ml
2	$La(N_2H_4)_2(2\text{-}C_{10}H_7COO)_3].2H_2O$	125µg/ml	62.5µg/ml
3	$[La(N_2H_4)_2\{C_{10}H_6(1\text{-}O)(2\text{-}COO)\}_{1.5}].3H_2O$	62.5µg/ml	62.5µg/ml
4	$[La(N_2H_4)_2\{C_{10}H_6(2\text{-}O)(1\text{-}COO)\}_{1.5}]$	62.5µg/ml	62.5µg/ml
5	$[La(N_2H_4)_2\{C_{10}H_6(3\text{-}O)(2\text{-}COO)\}_{1.5}].H_2O$	62.5µg/ml	125µg/ml
6	$[La(N_2H_4)_2\{2\text{-}C_{10}H_7OCH_2(COO)\}_3].3H_2O$	250µg/ml	250µg/ml

CONCULSION

Antibacterial activity of $La(N_2H_4)_2(1\text{-}C_{10}H_7COO)_3].2H_2O$, $[La(N_2H_4)_2\{C_{10}H_6(1\text{-}O)(2\text{-}COO)\}_{1.5}].3H_2O$ and $[La(N_2H_4)_2\{C_{10}H_6(2\text{-}O)(1\text{-}COO)\}_{1.5}]$ complexes was greater than $La(N_2H_4)_2(2\text{-}C_{10}H_7COO)_3].2H_2O$ $[La(N_2H_4)_2\{C_{10}H_6(3\text{-}O)(2\text{-}COO)\}_{1.5}].H_2O$ and $[La(N_2H_4)_2\{2\text{-}C_{10}H_7OCH_2(COO)\}_3].3H_2O$ complexes. The higher bacteria toxicity experienced by the compounds may be ascribed to the fact that the ligand and metal ions are more susceptible towards the bacterial cells.

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