ANTIBIOLOGICAL ACTIVITIES OF LANTHANUM COMPLEXES

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ABSTRACT

Synthesized lanthanum Naphthoic and Hydroxy Naphthoic complexes have been screened for antibacterial and anti fungal activity and results were compared with the activity of the un complexed antibiotic against tested against the bacterial species Staphylococcus aureus, Escherichia coli, Salmonella typhi and Bacillus subtilis by Agar cup method. Fungal species Aspergillus niger, Penicillium chrysogenum, Fusarium moneliforme and Aspergillus flavus by the posion plate method. The lanthanum complexes were found to be more potent against one or more bacterial species than the uncomplexed Penicillin and Gresiofulvin.

Key words - Staphylococcus aureus; Antibacterial activity; Anti fungal acitivity; Naphthoic Acid

INTRODUCTION

Hydrazones belongs to latent 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 come out as important class of nitrogen and oxygen or sulfur ligands particularly for transition and inner transition metal ions in the last two decades. The field of bioinorganic chemistry with hydrazone complexes has increased interest as biological important chemical compounds from aryl hydrazones are acting as enzyme inhibitors and also useful for pharmacological applications (Dharamaraj, Viswanathamurthi, & Natarajan, 2001). Metallic compounds act as a catalyst for conducting various chemical reactions and it's making different chemical complexes that are effective against bacteria, fungi, and many other microbes 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 estimate 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 bridge coordinating through hydrazide >C=O and nitrogen of >C=N. The Antibacterial and antifungal studies indicate an activity of the ligands on complexation (Agarwal et al 2009). Many natural products with the coumarinic moiety exhibit interesting biological and pharmacological properties. They are antibacterial, anti-HIV active (S. Hesse etal 2002) and antihelimenthic (B. H. Lee etal 1998)

The lanthanides have inhibitory activity against bacteria and that they are used in the medical field. 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). Venkatesh Mutalik et al 2011 explained the synthesis, characterization, fluorescent and antimicrobial properties of new Lanthanide(III) complexes obtained from coumarin Schiff base.

EXPERIMENTAL SECTION

Preparation 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 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 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. At pH 6, the metal solution was added and stirring the reaction mixture vigorously, microcrystalline solid formed immediately. Then the complex was filtered, washed with water, alcohol and then with ether and dried in a desiccators over anhydrous $CaCl_2$.

Collection and Storage of Test Organisms

The organisms used were clinical separates and antibacterial activity was measured by agar cup method (R. Cruickshank etal 1998). The bacterial cultures selected were, two gram negative cultures viz. Escherichia coli, Salmonella typhi and two Gram positive cultures viz. Staphylococcus aureu, Bacillus subtilis.

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).

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.

Sterilization of Forceps

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

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 Penicillin 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.

Results were recorded by measuring the zone of inhibition in millimeter (mm) using zone reader (Table- 1). Antifungal activity was performed by Poison plate method (H. N.Sheikh etal 2004). The medium used was Potato Dextrose Agar method (R. Cruickshank etal 1998). Aspergillus niger, Penicillium chrysogenum, Fusarium moneliforme, Aspergillus flavus were selected as test fungal cultures. Results were recorded (table-2) as moderate growth of fungi (++), reduced growth of fungi (+) and no growth of inoculated fungi (-) antifungal activity.

Minimum Inhibitory Concentration

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.

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 8^{th} tube was taken as control.
- 1ml of Muller Hinton Broth was transferred to all tubes except 6^{th} and 7^{th}.
- 0.5ml of broth was transferred to 6^{th} & 7^{th} tubes.
- 1ml of drug solution was added to 1^{st} tube and mixed well.
- From the 1^{st} tube transfer 1ml of solution to the 2^{nd} tube and was repeated up to 6^{th} tube.
- From the 6^{th} tube 0.5ml of solution was taken and transferred to 7^{th} 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 four bacteria such as Escherichia coli, Salmonella typhi, Staphylococcus aureu and Bacillus subtilis.

Penicillin was used as standard drug. The lanthanum complexes were evaluated for anti-bacterial and anti-fungal activity with different strains of bacteria and fungi. Results are shown in table-1 and table-2.

Table 1 Anti Bacterial Activity of Synthesized Complexes

COMPOUNDS	Zone of inhibition			
	E. coli	***S. typhi***	***S.aureus***	***B. subtilis***
$La(N_2H_4)_2(1\text{-}C_{10}H_7COO)_3].2H_2O$	11	15	17	12
$La(N_2H_4)_2(2\text{-}C_{10}H_7COO)_3].2H_2O$	12	16	18	11
$[La(N_2H_4)_2\{C_{10}H_6(1\text{-}O)(2\text{-}COO)\}_{1.5}].3H_2O$	15	21	19	16
$[La(N_2H_4)_2\{C_{10}H_6(2\text{-}O)(1\text{-}COO)\}_{1.5}]$	15	21	20	16
$[La(N_2H_4)_2\{C_{10}H_6(3\text{-}O)(2\text{-}COO)\}_{1.5}].H_2O$	19	24	19	17
$[La(N_2H_4)_2\{2\text{-}C_{10}H_7OCH_2(COO)\}_3].3H_2O$	9	13	9	11
SD*	20	25	21	18

SD* - Standard drug- Penicillin

The complexes were sensitive to S. typhi and S. aureus organisms with inhibitory zones within 9-24 mm. Higher activity observed against the gram negative bacteria S. typhi for $[La(N_2H_4)_2\{C_{10}H_6(3\text{-}O)(2\text{-}COO)\}_{1.5}].H_2O$ complex.

All have shown lesser activity against E. coli, S. aureus and B. subtilis compared with penicillin taken as standard. Higher activity observed against the bacteria Salmonella typhi for $[La(N_2H_4)_2\{C_{10}H_6(3\text{-}O)(2\text{-}COO)\}_{1.5}].H_2O$ complex. Antifungal activity observed against Aspergillus species was encouraging in comparison with Penicillium chrysogenum and Fusarium moneliforme.

Table 2 Anti Fungal activity

No.	Compounds	Growth of Fungi			
		A. niger	**P .chrysogenum**	**F. moneliforme**	**A. flavus**
1	$La(N_2H_4)_2(1\text{-}C_{10}H_7COO)_3].2H_2O$	+	++	+	+
2	$La(N_2H_4)_2(2\text{-}C_{10}H_7COO)_3].2H_2O$	+	-	++	+
3	$[La(N_2H_4)_2\{C_{10}H_6(1\text{-}O)(2\text{-}COO)\}_{1.5}].3H_2O$	-	++	+	-
4	$[La(N_2H_4)_2\{C_{10}H_6(2\text{-}O)(1\text{-}COO)\}_{1.5}]$	+	+	++	++
5	$[La(N_2H_4)_2\{C_{10}H_6(3\text{-}O)(2\text{-}COO)\}_{1.5}].H_2O$	-	-	-	-
6	$[La(N_2H_4)_2\{2\text{-}C_{10}H_7OCH_2(COO)\}_3].3H_2O$	+	++	-	+
	Gresiofulvin	-	-	-	-

Moderate growth (++), Reduced growth (+) and No growth (-) of fungi

Among all the complexes $[La(N_2H_4)_2\{C_{10}H_6(3\text{-}O)(2\text{-}COO)\}_{1.5}].H_2O$ complexe showed very good activity in antibacterial and antifungal activity 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 Penicillin. 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.

CONCULSION

Antibacterial and antifungal activity of$[La(N_2H_4)_2\{C_{10}H_6(3\text{-}O)(2\text{-}COO)\}_{1.5}].H_2O$complex was greater activity than $La(N_2H_4)_2(1\text{-}C_{10}H_7COO)_3].2H_2O$, $[La(N_2H_4)_2\{C_{10}H_6(1\text{-}O)(2\text{-}COO)\}_{1.5}].3H_2O$, $[La(N_2H_4)_2\{C_{10}H_6(2\text{-}O)(1\text{-}COO)\}_{1.5}]$, $La(N_2H_4)_2(2\text{-}C_{10}H_7COO)_3].2H_2O$ 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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