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A study of *in vitro* antibacterial activity of lanthanides complexes with a tetradentate Schiff base ligand

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PEER REVIEW

Peer reviewer

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Comments

This is a well written topical study with great potential in view of our losing battle with microbes. This appears to be one of the few papers to date showing the antibacterial potential of these compounds.
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ABSTRACT

Objective: To establish the antibacterial activity of lanthanides complexes with a tetradentate Schiff base ligand L. **Methods:** (N, N′-bis (1-naphthaldimine)-o-phenylenediamine) was prepared from the condensation of 2-hydroxy-1-naphthaldehyde with o-phenylenediamine in a molar ratio of 2:1. The antimicrobial activity of the resultant Ln (III) complexes was investigated using agar well diffusion and micro-broth dilution techniques; the latter was used to establish the minimum inhibitory concentrations for each compound investigated. **Results:** Most of Ln (III) complexes were found to exhibit antibacterial activities against a number of pathogenic bacteria with MICs ranging between 1.95-250.00 μg/mL. *Staphylococcus aureus* was the most susceptible bacterial species to $[LaL(NO_3)_2(H_2O)](NO_3)$ complex while *Shigella dysenteriae* and *Escherichia coli* required a relatively higher MIC (250 μg/mL). The complexes La (III) and Pr (III) were effective inhibitors against *Staphylococcus aureus*, whereas Sm (III) complex was effective against *Serratia marcescens*. On the other hand, Gd (III), La (III) and Nd (III) were found to be more potent inhibitors against *Pseudomonas aeruginosa* than two of commonly used antibiotics. The remaining Ln (III) complexes showed no remarkable activity as compared to the two standard drugs used. **Conclusions:** Tetradentate Schiff base ligand L and its complexes could be a potential antibacterial compounds after further investigation.

KEYWORDS
Antimicrobial, Biomolecules, Organics, Synthetic ligands

1. Introduction

Worldwide, infectious diseases are considered as number one cause of death accounting for approximately one-half of all deaths in tropical countries[1]. In industrialized nations, despite the progress made in the understanding of microbiology and their control, incidents of epidemics due to drug resistant microorganisms and the emergence of hitherto unknown disease-causing microbes, pose enormous public health concerns[1]. Perhaps it is not surprising to see these statistics in developing nations, but what may be remarkable is that infectious disease mortality rates are actually increasing in developed countries, such as the United States[2].

Recently, numerous antimicrobial drugs have been developed to kill or inhibit the growth of pathogenic microbes. However, the therapeutic efficiency of these drugs is still inadequate to attain the optimized therapeutic index[3]. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action because there has been an alarming increase in the incidence of new and reemerging infectious diseases[4].

The efficacy of a new molecule is determined by its ability to circumvent the development of resistance by the targeted bacteria and to expand the range of bacteria that can be treated with it[5]. To overcome the threat of antibiotic

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resistant bacteria, drug companies are in the process of development of new antibiotics and some have already reached clinical trials[6]. Many of these are semi-synthetic modifications of already existing antibiotics, including new beta-lactams, macrolides glycopeptides, quinolones and modifications of vancomycin. Others are entirely new, attacking previously unexploited chinks in the bacterial armor[7].

Drug resistance is a phenomenon that frequently impairs proper treatment of infections and cancer with chemotherapics. Intrinsic drug resistance relates to the failure of many microorganisms and tumours to respond to initial chemotherapy, while acquired drug resistance occurs when a microorganism or a tumour initially responds to chemotherapy but later relapses and appears to be strongly resistant to the original treatment[8]. Multidrug resistance in bacteria may be generated by one of two mechanisms. First, these bacteria may accumulate multiple genes, each coding for resistance to a single drug, within a single cell. This accumulation occurs typically on resistance plasmids. Second, multidrug resistance may also occur by the increased expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs[9,10]. Acquired resistance to antibiotics is currently steadily increasing in microorganisms. Therefore, much attention is being paid to the synthesis of novel biologically active compounds, which exhibit a broad spectrum of biological activity[11]. Complexations in many instances enhance their biological activity[12]. Thiosemicarbazones of heterocyclic carbonyl pyridine derivative have received special attention[13].

The aim of this study was to evaluate the antimicrobial activity of the synthetic lanthanides complexes with a tetradentate Schiff base ligand against some medically important bacteria and to investigate factors relevant to the biological activity of these compounds.

2. Materials and methods

2.1. Bacterial species

The clinical isolates used in this study were obtained from the Central Laboratories, Jordan Ministry of Health. These clinical isolates were *Shigella dysenteriae* (*S. dysenteriae*), *Escherichia coli* (*E. coli*), *Proteus vulgaris* (*P. vulgaris*), *Serratia marcescens* (*S. marcescens*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*).

2.2. Preparation of cultures for antimicrobial susceptibilities

Bacterial isolates were grown in 5 mL aliquots of Mueller-Hinton broth medium for 24 h at 37 °C. The inoculum density of each bacterial isolate was standardized with 0.5 McFarland turbidity standards. The suspension had a final inoculum of 5×10^7 cfu/mL.

2.3. Synthesis of Schiff base ligand L

The ligand L was prepared according to the method described by Ziyad A *et al*[14]. A 2.0 equivalent of 2-hydroxy-1-naphthaldehyde were condensed with a 1.0 equivalent of o-phenylenediamine in refluxing ethanol. Upon cooling, the yield was a crude orange product which was collected by filtration and washed with cold ethanol before being air-dried.

2.4. Synthesis of lanthanide complexes

One millimole (0.416 g) of the ligand L was dissolved in 10 mL chloroform. To this solution, 10 mL ethyl acetate solution of 1.0 mmol (0.433 g) $La(NO_3)_3(H_2O)_6$ were added in drop wise manner. The reaction mixture was stirred for 2 h at room temperature. The yellow precipitate formed was filtered, washed several times with ethyl acetate and chloroform, and then dried for 24 h in a vacuum at room temperature. All other Ln (III) complexes were prepared in a similar manner.

2.5. Agar diffusion method

Lanthanides complexes with a tetradentate Schiff base ligand antimicrobial activity was initially determined using the agar diffusion method[15]. Autoclave sterilized 20 mL aliquots of Mueller-Hinton agar were poured into 90 mm petri-dishes. Once these plates were cooled a bacterial lawn was prepared by spreading 100 μL from the above prepared bacterial suspension using sterile swabs. Wells of 6 mm in diameter were punched into the agar and filled with 100 μL of the antimicrobial under investigation at a concentration of 1 μg/mL[16]. The plates were then incubated at 37 °C for 24 h before zones of inhibition were measured using a caliper[15].

2.6. Micro-broth dilution minimum inhibition concentration

Antimicrobial activities of the ligand L and its Ln (III) complexes were estimated by the determination of minimum inhibitory concentration (MIC, μg/mL) using a micro-broth dilution method and following the guidelines of Hannan[16]. A stock solution of each antimicrobial in dimethylsulfoxide was prepared according to National Committee for Clinical Laboratory Standards guidelines[17]. The MIC was carried out in standard sterile 96 well flat bottom microtitre plates and the layout was designed so that each row covered the final antimicrobial dilution of 500.0-0.5 μg/mL with one control well.

Using sterilized micropipette, a 40 μL of the selected antimicrobial with the correct concentration was added to each well and another well was loaded with the same volume of control (DMSO solvent). Then a 150 μL of Mueller Hinton media was added to all wells, followed by a 10 μL of the respective bacterial suspension to give a final concentration of 5×10^7 cfu/mL in each well. The plates were sealed and incubated at 37 °C under atmospheric conditions. After 24 h incubation the microtitre plates were read using the ELIZA reader and the lowest concentration with optical density below that of the control was defined as the MIC.

3. Results

3.1. Agar diffusion method

Five of the eight Lanthanides complexes with a tetradentate Schiff base ligand in the concentration of 500 μg/mL were highly active against *S. aureus*. Lanthanides complexes with a tetradentate Schiff base ligand showed no activity against *S. dysenteriae* or *P. vulgaris*. The degree of

susceptibility of *P. aeruginosa*, *E. coli* and *K. pneumoniae* ranged from no activity to moderately activity for the Lanthanides complexes. Table 1 shows the results of agar well diffusion for all bacterial isolates.

Table 1

Agar well diffusion of the Schiff base ligand L and $[Ln(NO_3)_2L(H_2O)_x](NO_3)$ complexes against a number of bacteria.

Tested compounds	Gram (−) bacteria			Gram (+) bacteria			
	Sd	Ps	Pv	*E. coli*	Sm	Kp	Sa
L	N	++	N	++	N	N	N
$[DyL(NO_3)_2(H_2O)](NO_3)$	+	+	++	+	N	N	N
$[SmL(NO_3)_2](NO_3)$	N	+	N	+	++	N	+++
$[PrL(NO_3)_2(H_2O)](NO_3)$	N	++	N	++	N	N	+++
$[NdL(NO_3)_2](NO_3)$	N	++	N	++	N	++	+++
$[LaL(NO_3)_2(H_2O)](NO_3)$	N	++	N	++	N	++	+++
$[ErL(NO_3)_2(H_2O)](NO_3)$	N	++	N	++	N	++	+
$[GdL(NO_3)_2(H_2O)](NO_3)$	N	++	N	+	N	++	+++
Cephalexin	+++	++	++	+++	++	+++	+++
Cephradine	+++	++	++	+++	++	+++	+++

Sa: *S. aureus*; Ps: *P. aeruginosa*; Sd: *S. dysenteriae*; Pv: *P. vulgaris*; Sm: *S. marcescens*; Kp: *K. pneumonia*; N: non detected. Cephalexin and Cephradine are standard antibiotics.

−: No inhibition zone, inactive; +:1–5 mm inhibition zone, less active; ++: 6–10 mm inhibition zone, moderately active; +++: 10–15 mm inhibition zone, highly active.

3.2. MIC determination

The MIC results for each bacterial species tested are given in Table 2. It is clearly evident from this table that *in vitro* effectiveness of some ligands varies depending on the bacterial species tested. For instance, *S. aureus* consistently exhibited lower MIC as compared to other bacterial species at 1.95 μg/mL and 3.9 μg/mL for $[LaL(NO_3)_2(H_2O)](NO_3)$ and $[PrL(NO_3)_2(H_2O)](NO_3)$ synthetic complexes, respectively. In comparison, Gram negative bacteria such as *E. coli*, *P. aeruginosa* and *K. pneumoniae* required higher MIC for inhibition. In contrast, three bacterial isolates *S. dysenteriae*, *P. vulgaris* and *S. marcescens*, each was susceptible for only one synthetic ligand $[DyL(NO_3)_2(H_2O)](NO_3)$ with MIC of 250 μg/mL, $[DyL(NO_3)_2(H_2O)](NO_3)$ with MIC of 125 μg/mL and $[SmL(NO_3)_2](NO_3)$ with an MIC of 62.5 μg/mL respectively.

Table 2

MIC of the Schiff base ligand L and $[Ln(NO_3)_2L(H_2O)_x](NO_3)$ complexes against a number of bacteria (μg/mL).

Tested compounds	Gram (−) bacteria			Gram (+) bacteria			
	Sd	Ps	Pv	*E. coli*	Sm	Kp	Sa
L	N	63	N	63	N	N	N
$[DyL(NO_3)_2(H_2O)](NO_3)$	250	250	125	250	N	N	N
$[SmL(NO_3)_2](NO_3)$	N	250	N	250	63	N	16
$[PrL(NO_3)_2(H_2O)](NO_3)$	N	125	N	125	N	N	N
$[NdL(NO_3)_2](NO_3)$	N	63	N	63	N	125	8
$[LaL(NO_3)_2(H_2O)](NO_3)$	N	63	N	125	N	125	2
$[ErL(NO_3)_2(H_2O)](NO_3)$	N	125	N	125	N	125	250
$[GdL(NO_3)_2(H_2O)](NO_3)$	N	32	N	250	N	125	16
Cephalexin	8	125	125	16	125	8	8
Cephradine	16	125	125	16	125	16	8

Sa: *S. aureus*; Ps: *P. aeruginosa*; Sd: *S. dysenteriae*; Pv: *P. vulgaris*; Sm: *S. marcescens*; Kp: *K. pneumonia*; N: non detected. Cephalexin and Cephradine are standard antibiotics.

4. Discussion

As far as we know, this is the first study which investigates the antibacterial activity of tetradentate Schiff base ligand complexes. The MIC values were determined following the methods used by other investigators and essentially followed the guidelines described by Hannan PCT[18]. Although a limited number of isolates were tested, some prediction can be made about possible outcome of treatment efficacy since, in hospitals, treatment is based on tests on one clinical isolate. For *S. aureus* which is the only Gram positive isolate among the tested bacterial species, La (III), Pr (III) and Nd (III) were clearly the most effective *in vitro*, whereas Er (III) gave the lowest MIC values for the same microorganism. Gram negative bacteria were less susceptible to these synthetic complexes as effective inhibition required MICs ranging from 32 to 250 μg/mL. *S. dysenteriae* showed a limited susceptibility to one ligand and that is Dy (III) with an MIC 250 μg/mL. Unexpectedly *P. aeruginosa* which is considered as one of the most recalcitrant bacterial species to various antimicrobials were susceptible to all ligand complexes in the range of 32 μg/mL for Gd (III) and 250 μg/mL for Dy (III). *E. coli* was also susceptible to all ligand complexes with relatively higher MIC than *P. aeruginosa*, which gave an MIC of 63–250 μg/mL.

All Ln (III) complexes showed moderate, poor and even non-detectible antimicrobial activity against the Gram-negative bacteria tested. The higher activity of the complexes compared to free ligand may be attributed to chelation[19] which reduces polarity of the metal ion by partial sharing of the positive charge with donor atoms of the ligand. This increases the lipophilic character, favouring the permeation through lipid layers of the bacterial membrane. Moderate activity observed against the gram negative bacteria can be explained by considering the effect on lipopolysaccharide (LPS), a major component of the surface of gram negative bacteria[20,21]. LPS is an important entity in determining the outer membrane barrier function and the virulence of gram negative pathogens. The Schiff base can penetrate the bacterial cell membrane by coordination of metal ion through oxygen or nitrogen donor atom to LPS which leads to the damage of outer cell membrane and consequently inhibits growth of the bacteria.

Most of the Ln (III) complexes showed higher activity against both types of bacteria than the free ligand L. This enhancement in the activity can be explained on the basis of chelation theory[22]. Chelation reduces the polarity of the Ln (III) ions due to the partial sharing of lanthanides positive charge with the imine and oxygen donor atoms and possibly the electron delocalization over the whole chelate ring system[23]. Therefore, chelation increases the lipophilic nature of the central metal atom, which enhances the penetration of the complexes into the lipid membrane of the microorganism cell wall and thus raising the activity of the complex and restricts the further growth of the organism[24]. On comparing the antimicrobial activity of the ligand L and its Ln (III) complexes with the standard Cephalexin and Cephradine (antibacterial agents), several observations were made; La (III) and Pr (III) complexes were more active against *S. aureus*, Sm (III) complex was more efficient against *S. marcescens*, and Gd (III), La (III) and Nd (III) were more inhibitory against *P. aeruginosa* than the standard antibiotics; the remaining Ln (III) complexes show no remarkable activity compared with the slandered drugs, and the Gram-positive bacteria were much more susceptible

than the Gram-negative bacteria towards the tested Ln (III) complexes and that could be attributed to the complex structure of Gram negative bacteria.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The battle against infectious disease is in danger of being lost primarily as a result of drug resistance which means that in the not too distant future we will have run out of antimicrobials to treat new and existing infections. This paper contributes to the search for new and novel compounds.

Research frontiers

Studies are being carried out to assess the antimicrobial activity of synthetic lanthanides complexes with a tetradentate Schiff base ligands.

Related reports

As far as can be gathered from a brief look at the literature, the group at Al Balqa are presently the leading researchers in this area (Taha *et al.* 2011).

Innovations and breakthroughs

This appears to be one of the few papers to date showing the antibacterial potential of these compounds.

Applications

There are numerous possible applications of this work both for use in the treatment of infectious disease animals and humans. Clearly more isolates need to be tested and tolerance in humans and animals should be carefully examined.

Peer review

This is a well written topical study with great potential in view of our losing battle with microbes. This appears to be one of the few papers to date showing the antibacterial potential of these compounds.

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