New Ce(III) Complex Compounds of 2-(N,N-dimethylbiguanidil)-penta-1,5-dioic Acid with Biological Activity

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The paper present the biological properties, total antioxidant capacity and antimicrobial activity against different Gram positive and Gram negative strains and fungi, of some new Ce(III) complex compounds with Schiff base as ligands; the ligands were obtained by condensation between α-ketoglutaric acid and N,N-dimethylbiguanide (base and hydrochloride), in well-established condition. The new synthesized ligands were obtained as hydrochloride salt (2-(N,N-dimethylbiguanidil)-penta-1,5-dioic hydrochloride acid, L1) and also in neutral form (2-(N,N-dimethylbiguanidyl)-penta-1,5-dioic acid, L2); the new ligands and theirs Ce(III) complex compounds have been physico-chemically characterized by elemental chemical analysis, molar electrical conductivity, FT/IR, UV-Vis spectrometry and NMR. The surface of the ligands and complex compounds [Ce(L1)(NO3)3(OH2)4] and [Ce(L2)(NO3)3(OH2)4]HCl powders, were investigated in terms of morphology by optical microscopy reflection in the scan. The biological activity of new complex compounds [Ce(L1)(NO3)3(OH2)4] and [Ce(L2)(NO3)3(OH2)4]HCl, were L1 = 2-(N,N-dimethylbiguanidil)-penta-1,5-dioic hydrochloride acid and L2 = 2-(N,N-dimethylbiguanidil)-penta-1,5-dioic acid was increased compared to that of the new ligands and reactants used for synthesis.

Keywords: Ce(III) complex compounds, 2-(N,N-dimethylbiguanidil)-penta-1,5-dioic acid, biological activity

From literature data are known the biological and pharmacological applications of α-ketoglutaric acid (2-oxoglutaric acid), (fig. 2). It is known as intermediary in carbohydrate metabolism [1-4], as substrate for glutamate-dehydrogenase [5-7], as reagent for tryptophan analysis [8, 9], as a source of energy needed to bacterial metabolism (by introducing it into the culture environment of some pathogenic strains such as Pseudomonas aeruginosa and Corynebacterium) and also as a supplement to improve the ability of the liver detoxifier [9, 10]. On the other side, the properties and practical applications of N,N-dimethylbiguanide (fig. 1) as antidiabetic drug (metformin) are well known [11-22].

In this paper we propose the synthesis of a new organic ligand obtained in two form: 2-(N,N-dimethylbiguanidil)-penta-1,5-dioic hydrochloride acid (L1) and 2-(N,N-dimethylbiguanidyl)-penta-1,5-dioic acid (L2) and some new Ce(III) complex compounds with L1 and L2. The new complexes [Ce(L1)(NO3)3(OH2)4] and [Ce(L2)(NO3)3(OH2)4]HCl, have been characterized by elemental chemical analysis, molar electrical conductivity, FT/IR, NMR and UV-Vis spectrometry. Different biological activity tests [23-26] have shown an increased antioxidant and antimicrobial activity. In the literature, there are a limited number of works relating to these issues [27-34].

Experimental part

Synthesis of ligands (Schiff base type)

The ligands were obtained by condensation under refluxing between a mixture of α-ketoglutaric acid with N,N-dimethylbiguanide hydrochloride salt, in molar ratio 1:1 (L1, fig. 3a) and α-ketoglutaric acid with N,N-dimethylbiguanide base, in molar ratio 1:1 (L2, fig. 3b); methanol was used as solvent, at pH = 8. The new ligands...
L₁ and L₂ are polycrystalline powders, colored, insoluble in water, ethyl ether, chloroform, benzene, hexane, partially soluble in dimethylformamide, acetonitrile and soluble in dimethylsulfoxide, acetone, methyl alcohol. The structures of L₁ and L₂ were determined by elemental chemical analysis (table 1) and NMR and FT/IR spectroscopy (table 3).

**Synthesis of Ce(III) complex compounds with 2-(N,N-dimethylbiguanidyl)-penta-1,5-dioic acid**

Complex compounds [Ce(L₁)(NO₃)₃(OH₂₄)]HCl, [Ce(L₂)(NO₃)₃(OH₂₄)]HCl, where L₁ and L₂ are 2-(N,N-dimethylbiguanidyl)-penta-1,5-dioic acid and respectively 2-(N,N-dimethylbiguanidyl)-penta-1,5-dioic acid, have been obtained from Ce(NO₃)₃·6H₂O p.a. (Fluka-Aldrich) and the ligands, L₁ and L₂. Synthesis was conducted under refluxing, in methyl alcohol as solvent, at ambient temperature; both of them are colored, stable to light and to air, soluble in dimethylsulfoxide, acetone, methyl alcohol.

<table>
<thead>
<tr>
<th>Compound</th>
<th>C% found</th>
<th>C% calculated</th>
<th>A [Ω·cm²·mol⁻¹]</th>
<th>Type of electrolyte/Aspect</th>
</tr>
</thead>
<tbody>
<tr>
<td>(L₁) 2-(N,N-dimethylbiguanidyl)-penta-1,5-dioic acid</td>
<td>36.72</td>
<td>36.72</td>
<td>36.80</td>
<td>Electrolyte/powder, red-brownish</td>
</tr>
<tr>
<td>(L₂) 2-(N,N-dimethylbiguanidyl)-penta-1,5-dioic acid</td>
<td>41.93</td>
<td>41.93</td>
<td>42.02</td>
<td>Electrolyte/powder, yellowish</td>
</tr>
<tr>
<td>(1) [Ce(L₁)(NO₃)₃(OH₂₄)]HCl</td>
<td>22.20</td>
<td>22.20</td>
<td>22.27</td>
<td>Nonelectrolyte/powder, yellow-orange</td>
</tr>
<tr>
<td>(2) [Ce(L₂)(NO₃)₃(OH₂₄)]HCl</td>
<td>20.65</td>
<td>20.65</td>
<td>20.71</td>
<td>Nonelectrolyte/powder, brown</td>
</tr>
</tbody>
</table>

**Methods and equipment for the physico-chemical and biological characterization of the new synthesized compounds**

The new ligands and complexes were characterized, as follows:

- elemental chemical analysis was done by samples disaggregation with hydrogen peroxide in ammonia. Determination of 4' trivalent ions as oxides and of the anions was performed by gravimetric methods [35];
- nitrogen determination was done by microcombustion (Dumas method) and carbon by Schoniger method [35];
- FT-IR spectra were registered with a Jasco 620 spectrophotometer, PC coupled, solid samples in KBr pill, on 4000 - 400 cm⁻¹ field;
- electronic spectra were registered through diffuse reflection technique with a Jasco V-570 UV/VIS/NIR spectrophotometer, PC coupled, on 190-2300 nm spectral field, using MgO standard;
- 1H-NMR spectra were registered in dimethylsulfoxide at 22°C, with Bruker Avance apparatus, 400MHz;
- molar electrical conductivity were registered with a Radelkis KFT conductometer, 0.1μS 0.5μS field;
- the surface of ligands L₁ and L₂ and complex compounds (1) and (2) as powders, were morphologically investigated by scanning optical microscopy in reflection. The size powders were analyzed with an optical microscope in reflection, Buehler Germany, with the objective degree of progressive increase Ob 25x, 60x, 100x and 10x, optical Oc with the scale [cm] and [mm] and flexible full system acquisition, analysis and archiving of image capture, Ommnet Enterprise;
- for antioxidative activity determination by chemiluminescence method, a TD 20/20 Turner Design USA chemiluminometer was used, PC coupled, provided with 1.5 mL enclosed glass cuvettes; the generating chemiluminescence system was luminol (10⁻⁴M) - H₂O₂ (10⁻⁴M) in Tris-HCl buffer solution, pH=8.4, final volume 1 mL, with DMSO p.a. (Merck, Germany) as solvent for the standard luminol. Samples concentration were in 10⁻⁵M in DMSO. The standard luminol, present CL signal inhibition Iᵢ=3770 (a) [44, 45].
- Antimicrobial activity of new ligands and complex compounds have been determined on various pathogenic and/or conditioned pathogenic bacterial and fungal strains, by measuring the minimum inhibition diameter of bacterial and fungal stains (μg/mL), in the aim to estimating the minimum inhibitory concentration of the substance (MIC) and the diffusion power of substances in the agar culture. Immediately after contact between each substance and microbial strain, the viability or their mobility is influenced by the compounds concentrations. Filter paper discs (φ = 6 mm) were impregnated with 10 μL solution of 10⁻⁵M in acetone of the ligands, complexes and reactants, and then placed on agar surface inoculated with bacterial and fungal strain. Acetone as solvent was used to dissolve the compounds due to its fast evaporation, so that the effect on microbial strain is only due to the tested compound. The amounts of the compound/disc were between 10 and 11 mg/disc. Using disc diffusion technique, the complexes [Ce(L₁)(NO₃)₃(OH₂₄)]HCl, [Ce(L₂)(NO₃)₃(OH₂₄)]HCl, ligands L₁, L₂, and reactants N,N-dimethylbiguanide, α-ketoglutaric acid, Ce(NO₃)₃, 6H₂O were screened for antibacterial and antifungal activity, against five bacterial strains Gram positive and Gram negative species, Streptococcus β-haemolytic group A, Streptococcus β-haemolytic group B, Proteus mirabilis, Escherichia coli, Staphylococcus coagulase positive and on two fungal strains, Candida albicans and Saccharomyces cerevisiae, growing on agar. The plates were incubated at 37°C, 24 h for the bacteria and 72 h for the fungis strains. The inhibition zone [mm] of the microbial strain, was measured [46].

**Results and discussions**

**Morphological analysis**

The new ligands (L₁), (L₂) and complexes (1), (2) tend to form conglomerates (with spherical shapes, oblong or acicular), as follows:

- L₁ (fig. 4) is presented as well-developed crystals, transparent, colored red-brownish, with rounded edges;
is hygroscopic at ambient temperature and has a tendency to form glues;
- $L^2$ (fig. 5) is presented in the form of crystal yellowish-colored transparent clearly defined, with edges and corners; it is non-hygroscopic at ambient temperature and has a slight tendency to agglomerate;
- complex $[\text{Ce}(L^2)(\text{NO}_3)_3(\text{OH}_2)_4]$, 1, (fig. 6) is presented in the form of yellow-orange semi-crystals, well-developed, with edges and corners; it is non-hygroscopic at ambient temperature and has a slight tendency to agglomerate;
- complex $[\text{Ce}(L^1)(\text{NO}_3)_3(\text{OH}_2)_4] \cdot \text{HCl}$, 2, (fig. 7) is presented in the form of brown semi-crystals, with rounded edges; it is hygroscopic at ambient temperature and has a tendency to form glues.

**Electronic spectra**

To obtain information on the stereochemistry of metallic ion in complex compounds the electronic spectra by diffuse reflection technique in UV-Vis-NIR 200 - 2300 nm, have been recorded (table 2) [36-39].

Ligands $L^1$ and $L^2$ present two intensive bands at 224 nm, 248 nm ($L^1$) and 230 nm, 254 nm ($L^2$) and two low intensity bands at 1178 nm, 1710 nm ($L^1$), respectively 1028 nm, 1176 nm ($L^2$). The electronic spectra registered for complex compounds 1 and 2 show similar shapes in terms of number and position of absorption bands except the bands in the visible area. For complex compounds $[\text{Ce}(L^2)(\text{NO}_3)_3(\text{OH}_2)_4]$, 1 and $[\text{Ce}(L^1)(\text{NO}_3)_3(\text{OH}_2)_4] \cdot \text{HCl}$, 2 the presence of intensive bands at 274 nm and 272 nm (36,496 and 36,764 cm$^{-1}$ respectively), with slight movements of values at higher wavelength was observed. It is noted the presence of two weak bands in the near-infrared, at 1416 nm to 1716 nm and shoulder, for compound 1, at 1386 nm and 1918 nm for compound 2. These bands, easily moved to the ligands in the free state, is due to the presence of $L^1$ and $L^2$ in the coordination sphere.

**Infrared spectra**

For information about the ligands $L^1$ and $L^2$ coordinating manner and the position of anions $\text{NO}_3^-$ in the complex compounds $[\text{Ce}(L^2)(\text{NO}_3)_3(\text{OH}_2)_4]$, 1 and $[\text{Ce}(L^1)(\text{NO}_3)_3(\text{OH}_2)_4] \cdot \text{HCl}$, 2, infrared spectra at 4000 - 400 cm$^{-1}$ were recorded (table 3). The presence of absorption bands at 3400 - 3100 cm$^{-1}$ were attributed to the stretching vibration $\nu$ (NH) and $\nu$ (OH). Absorption bands in the field 1550 - 1590 cm$^{-1}$, were attributed to vibration -COOH group, proving that it is not participating in coordination. Absorption bands present at 1630 - 1650 cm$^{-1}$ were assigned to vibration of coordinated imino- group. For both compounds (1) and (2), absorption band from 820 - 830 nm were attributed to $\text{NO}_3^-$ which is inner the coordination sphere [40-43].

**NMR spectra**
The comparative 1H-NMR spectra study of the condensation ligands L1 and L2 with the complex compounds [Ce(L1)(NO3)3(OH2)4], 1 and [Ce(L2)(NO3)3(OH2)4]HCl, 2 are allowed to obtain information of the structure, based on magnetic protons differentiation, as a result of different electric molecule neighborhoods. In the 1H–NMR spectra recorded in DMSO - D6 at room temperature, for the new ligands L1 and L2 and reactants, signals 10.09 (s, COOH), 3.17 (s, 6H, 2CH3), 3.47 (s, 1H, NH), 2.96-2.98 (m, 4H, 2CH2) were observed; similar signals values for the complex compounds [Ce(L1)(NO3)3(OH2)4], 1 and [Ce(L2)(NO3)3(OH2)4]HCl, 2 were observed, which confirm the presence of L1 and L2 in the coordination sphere of 1 and 2 respectively.

According to the presented results, the following structures are proposed for the complexes [Ce(L1)(NO3)3(OH2)4], 1 and [Ce(L2)(NO3)3(OH2)4]HCl, 2 (fig. 8).

**Antioxidative activity**

Antioxidative activity by chemiluminescence method (CL) was quantified by calculating CL signal inhibition depending on time (1), in the presence of ligands L1, L2 and complexes 1 and 2 compared with α-ketoglutaric acid and N,N-dimethylbiguanide, where: % AA - antioxidative activity; I_o - chemiluminescent intensity signal of witness (luminol) at t = 5 d; I_p - chemiluminescent intensity signal in the presence of sample at t = 5 s.

Comportative analysis of CL signal inhibition values in time, of the ligands L1, L2 and complexes 1 and 2 compared with α-ketoglutaric acid and N,N-dimethylbiguanide base, N,N-dimethylbiguanide hydrochloride and α-ketoglutaric acid, at the concentration of 10−3 M, (table 4), present the following specificity:
- high values of CL signal inhibition for the complexes [Ce(L1)(NO3)3(OH2)4], 1 and [Ce(L2)(NO3)3(OH2)4]HCl, 2 show an intense antioxidative capacity, which is much higher than the one of the free ligands L1 and L2 or reactants (N,N-dimethylbiguanide and α-ketoglutaric acid) used as raw materials;
- CL signal inhibition analysis values on different ligands show a strong antioxidative capacity of the ligands L1 (AA% = 82.9%) and L2 (AA% = 87.3%), higher compared to reactants N,N-dimethylbiguanide hydrochloride (AA% =
Table 5
CL SIGNAL INHIBITION IN TIME FOR COMPLEXES (1) [Ce(L2)(NO3)3(OH2)4] AND (2) [Ce(L1)(NO3)3(OH2)4](HCl), at the concentration of 10^{-5}M

<table>
<thead>
<tr>
<th>Complex compound</th>
<th>Concentration (M)</th>
<th>AA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) [Ce(L2)(NO3)3(OH2)4]</td>
<td>10^{-6}</td>
<td>91.00</td>
</tr>
<tr>
<td></td>
<td>10^{-9}</td>
<td>94.00</td>
</tr>
<tr>
<td>(2) <a href="HCl">Ce(L1)(NO3)3(OH2)4</a></td>
<td>10^{-5}</td>
<td>84.70</td>
</tr>
<tr>
<td></td>
<td>10^{-9}</td>
<td>87.00</td>
</tr>
</tbody>
</table>

Table 6
MICROBIOLOGICAL ACTIVITY OF THE COMPLEX COMPOUNDS, LIGANDS AND REACTANTS

<table>
<thead>
<tr>
<th>Microbial strain</th>
<th>Microbial culture inhibition diameter [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Complex compound (1)</td>
</tr>
<tr>
<td>Gram positive bacteria</td>
<td>Streptococcus β-haemolytic group A</td>
</tr>
<tr>
<td></td>
<td>Streptococcus β-haemolytic group B</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus coagulase positive</td>
</tr>
<tr>
<td>Gram negative bacteria</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td></td>
<td>Proteus mirabilis</td>
</tr>
<tr>
<td>Fungi</td>
<td>Candida albicans</td>
</tr>
<tr>
<td></td>
<td>Saccharomyces cerevisiae</td>
</tr>
</tbody>
</table>

The obtained results (table 6) allowed to considering the tested substances as antibacterial and/or antifungal agents, as follows: • I° category - does not affect microbial strain growth; • II° category - inhibits the microbial strain growth with inhibition diameter range: \( \varnothing \) = 0.1 - 10 mm; • III° category - inhibits the microbial strain growth with inhibition diameter range: \( \varnothing \) > 10 mm.

The results emphasize specific microbial activities for the complex compounds [Ce(L2)(NO3)3(OH2)4], 1 and [Ce(L1)(NO3)3(OH2)4](HCl), 2, present antibacterial activity on Streptococcus β-haemolytic group A, Streptococcus β-haemolytic group B and Staphylococcus coagulase positive, Escherichia coli and Proteus mirabilis with inhibition diameter = 0.1-10 mm; No antifungal activity on

Microbiological activity

81%), N,N-dimethylbiguanide base (AA% = 82%) and α-ketoglutaric acid (AA% = 81.2%), that are almost similar.

- at different concentrations 10^{-5}M and 10^{-9}M, complexes [Ce(L^2)(NO3)3(OH2)4], 1 and [Ce(L^1)(NO3)3(OH2)4](HCl), 2, present different anti-oxidative capacity values. For the complex 1, an increased value of antioxidative capacity at the concentration of 10^{-9} M (AA% = 94%), that the one at the concentration of 10^{-5} M (AA% = 91%). The same situation is for the complex 2, the concentration of 10^{-9} M (AA% = 87%) shows an increased antioxidative capacity compared with the one at the concentration of 10^{-5} M (AA% = 84.7%) (table 5, fig. 9);

- by coordination with Ce(III) of the two condensation ligands L^1 and L^2, an increased antioxidative activity of the new complexes 1 and 2, at both concentrations 10^{-5} M and 10^{-9} M, were observed.
Saccharomyces cerevisiae and Candida albicans were observed;
- for ligands (L1) and (L2), no antimicrobial activity on Escherichia coli, Proteus mirabilis, Streptococcus β-haemolytic group A, Streptococcus β-haemolytic group B, Staphylococcus coagulase positive, Saccharomyces cerevisiae and Candida albicans were observed;
- for N,N-dimethylbiguanide base and N,N-dimethylbiguanide hydrochloride, no antimicrobial activity Streptococcus β-haemolytic group A, Streptococcus β-haemolytic group B, Staphylococcus coagulase positive, Escherichia coli, Proteus mirabilis, Saccharomyces cerevisiae and Candida albicans were registered;
- α-ketoglutaric acid, presents antibacterial activity on Streptococcus β-haemolytic group A, Streptococcus β-haemolytic group B, Staphylococcus coagulase positive, Escherichia coli and Proteus mirabilis, with inhibition diameter $\varnothing = 0.1-10$ mm; No antimicrobial activity on Saccharomyces cerevisiae and Candida albicans were observed;
- Ce(NO$_3$)$_3$.6H$_2$O, presents antibacterial activity on Escherichia coli, Proteus mirabilis, Streptococcus β-haemolytic group A, with inhibition diameter $\varnothing = 0.1-10$ mm; No antimicrobial activity on Streptococcus β-haemolytic group B, Staphylococcus coagulase positive, Saccharomyces cerevisiae and Candida albicans were registered.

Conclusions

New condensation ligands 2-(N,N-dimethylbiguanidiido)-penta-1,5-dioic hydrochloride acid, L$_1$, 2-(N,N-dimethylbiguanidil)-penta-1,5-dioic acid, L$_2$ and new complex compounds [Ce(L$_2$)(NO$_3$)$_2$(OH$_2$)$_4$]HCl, 1 and [Ce(L$_1$)(NO$_3$)$_2$(OH$_2$)$_4$]HCl, 2, have been synthesized and characterized by elemental chemical analysis, molar electrical conductivity, FT/IR, NMR and UV-Vis spectrometry. Powders size of the new compounds, in terms of morphology by optical microscopy reflection in the scan, was investigated.

Physico-chemical characterization put into evidence the proposed molecular structures: [Ce(L$_2$)(NO$_3$)$_2$(OH$_2$)$_4$]HCl, 1 and [Ce(L$_1$)(NO$_3$)$_2$(OH$_2$)$_4$]HCl, 2.

Using chemiluminescence method, an increased antioxidative activity for the new complex compounds 1 and 2, at different concentrations, compared with the one of the ligands L$_1$, L$_2$ and reactants N,N-dimethylbiguanide base, N,N-dimethylbiguanide hydrochloride and α-ketoglutaric acid were observed.

Microbiological tests conducted on seven microbial strains have shown that the new complexes 1 and 2 emphasize an activity comparable to that of the reactant α-ketoglutaric acid, only bactericidal effect against strains of Streptococcus β-haemolytic group A, Streptococcus β-haemolytic group B, Staphylococcus coagulase positive, Escherichia coli and Proteus mirabilis, with an inhibition diameter of $\varnothing = 2 - 9$ mm, more active against Gram positive bacteria and no antifungal activity. The complex 1 shows the highest antibacterial activity; we propose II$^{nd}$ category classification – as antimicrobial agent.

The condensation ligands L$_1$, L$_2$ and reactants N,N-dimethylbiguanide base and N,N-dimethylbiguanide hydrochloride, there are no antibacterial and antifungal activity against all the microbial strains tested; we propose I$^{st}$ category classification – as antimicrobial agent.

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