High antioxidative potential and low toxic effects of selenosemicarbazone metal complexes

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Abstract: Novel metal-based compounds with therapeutic potential have become the subject of intense investigations in inorganic chemistry and biomedical science. Recently, strong dose-dependent cytotoxic activities of selenosemicarbazone metal complexes against several human cancer cell lines were demonstrated. The aim of the present study was to investigate in vitro antioxidative potential of Ni(II), Cd(II) and Zn(II) selenosemicarbazone complexes. All three investigated complexes exhibited high 2,2’-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation (ABTS•+) scavenging capacity, comparable with ascorbic acid. In an acute toxicity study, administration of the compounds was performed orally to mice at single doses. The mice were observed for clinical signs, body weight effects and mortality for 14 days, after which they were sacrificed for gross organ necropsy. The body weight did not vary after administration, and the autopic analysis failed to show appreciable macroscopic alterations of internal organs. Generally, the compounds exhibited low toxic effects as required for further in vivo therapeutic studies.

Keywords: Ni(II), Cd(II) and Zn(II) complexes; selenosemicarbazones; antioxidative activity; in vivo toxicity.

INTRODUCTION

Chalcogensemicarbazones are condensation derivates of chalcogensemicarbazides and carbonyl compounds. Interest in (thio)semicarbazone ligands has been driven, in part, by potentially beneficial biological activity of ligands and their metal complexes, including, antifungal, antimicrobial, anticancer, anti-inflammatory and antiviral activities.1–4 However, total number of selenosemicarbazones and their metal complexes is much smaller in comparison to the corresponding O and S analogues, although results of few comparative studies

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indicated that isosteric replacement of other chalcogen atoms with selenium resulted in a much more active compounds.\textsuperscript{5–11} The biological action of chalcogenemicarbazones is attributed to their chelating properties.\textsuperscript{1–4}

Our research group has been engaged in the systematic investigation of selenosemicarbazones derived from N-heteroaromatic aldehydes and ketones, in order to determine the driving force behind the coordination behaviour of this type of ligands, as well as to explore their biological activity. Similar to their sulphur analogues, selenosemicarbazones exhibit various binding modes with d-metals. Selenosemicarbazones of aliphatic and aromatic carbonyl compounds can act as mono- or bidentate ligands, but when N-heteroaromatic carbonyl compound is used for synthesis of selenosemicarbazones, the coordination capacity can be extended by the presence of additional nitrogen atom suitable for chelation. Accordingly, 2,6-diacetylpyridine bis(selenosemicarbazone) (H\textsubscript{2}dapsesc) is coordinated as pentadentate, via both selenium atoms, pyridine and both imine nitrogen atoms in Cd(II) and Zn(II) complexes (Scheme 1),\textsuperscript{12} while during the formation of the Ni(II) complex, elimination of hydrogen selenide from one side chain of the H\textsubscript{2}dapsesc ligand occurred.\textsuperscript{12} In the resulting Ni(II) complex, the modified ligand 2-{1-[6-(1-selenosemicarbazonoethyl)-2-pyridyl]ethylidene}-hydrazine carbonitrile (H\textsubscript{2}hcn) is tetradentately coordinated (Scheme 1).

Over-production of activated oxygen species, generated by normal metabolic processes, is considered to be the main contributor of oxidative damage in biomolecules, thus inducing a broad spectrum of different diseases, in particular cardiovascular, neurodegenerative disease and cancer.\textsuperscript{13} It is known that oxidative stress plays a multistep role in carcinogenesis, through a process of both cell mutation and proliferation. Although most cancer cells exhibit elevated oxidative stress with increased metabolic activity and the production of reactive oxygen species (ROS), the mechanism of action of many cancer chemotherapeutic drugs involves ROS-mediated apoptosis. For example, the classic anticancer agents cisplatin and adriamycin appear to produce ROS at excessive levels, resulting in DNA damage and cell death.\textsuperscript{14} Most thiosemicarbazone complexes contain redox
metal ions that potentially can activate O$_2$ and generate OH· radicals. However, there are data that support but also data that exclude this activity as function of the nature of the ligand and the metal centre.\textsuperscript{4} The potential value of natural and synthetic antioxidants has already prompted scientists to search for cooperative effects of compounds which improve antioxidant activity and cytotoxicity.\textsuperscript{15} Previous investigations showed that selenosemicarbazone complexes exhibited strong dose-dependent cytotoxic activity against a panel of several human tumour cell lines, and this effect was comparable to that of cisplatin.\textsuperscript{16–19} This study was undertaken with the aim to investigate the \textit{in vitro} antioxidative potential of Ni(II), Cd(II) and Zn(II) selenosemicarbazone complexes. The analysis of the toxicity level of chemical compounds is one of the most important steps required for further biological studies.\textsuperscript{20} Bearing in mind the toxicity from high Se intake and that metal ions are generally toxic at high-dose levels, the second aim of the present study was to evaluate the acute oral toxicity of these novel metal-based compounds.

**EXPERIMENTAL**

**Materials and methods**

2,6-Diacetylpyridine (dap, 99 %), was obtained from Acros Organics, while Ni(CH$_3$COO)$_2$·4H$_2$O (\textit{purum p.a.} ≥ 99.0 % KT), Zn(CH$_3$COO)$_2$·2H$_2$O (\textit{purum p.a.} ≥ 99.0 % KT) and Cd(CH$_3$COO)$_2$·2H$_2$O (\textit{purum p.a.} ≥ 99.0% KT) were obtained from Fluka. Dimethyl sulphoxide (DMSO dried, ≥ 99.5 %) was obtained from Merck (Darmstadt, Germany). 2,2’-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS, anal. grade) and vitamin C (ascorbic acid,\textit{ p.a}) were purchased from Serva.

Elemental analyses (C, H, N) were performed by standard micro-methods using an ElementarVario ELIII C.H.N.S=O analyzer. The IR spectra were recorded on a Perkin–Elmer FT-IR 1725X spectrometer in the region 4000–400 and 400–200 cm$^{-1}$ as CsI tablets. Molar conductivities were measured at room temperature on a digital conductivity-meter Jenway-4009. The NMR spectra (in DMSO-\textit{d$_6$}) were obtained using a Varian Gemini 2000 instrument (\textit{1H} at 200 MHz, \textit{13C} at 50.3 MHz).

**Synthesis of the tested compounds**

The ligand H$_2$dapsesc was synthesized as described previously\textsuperscript{12} by the reaction of dap and selenosemicarbazide in the molar ratio 1:2. The complexes 1–3 (Scheme 1) were synthesized by adding an aqueous solution of Zn(CH$_3$COO)$_2$·2H$_2$O, Cd(CH$_3$COO)$_2$·2H$_2$O or Ni(CH$_3$COO)$_2$·4H$_2$O, respectively, into a suspension of H$_2$dapsesc in EtOH.\textsuperscript{12} The Zn(II) and Cd(II) complexes were purified by vapour diffusion using DMSO as the inner solvent and EtOH as the outer solvent. The purity of the synthesized compounds was tested by elemental analysis. The IR and NMR spectroscopy data, as well as the molar conductivity values were in good agreement with previously published data.\textsuperscript{12}

**Analytic and spectral data for the ligand and 1–3**

$H_2$dapsesc. Yield: 0.63 g (93 %); Anal. Calcd. for C$_{11}$H$_{15}$N$_7$Se (FW: 403.20): C, 32.77; H, 3.75; N, 24.32 %. Found: C, 32.96; H, 3.70; N, 23.79 %. IR (KBr, cm$^{-1}$): 3460 (s), 3375(s), 3333 (s), 3249 (s), 3147 (s), 1591 (vs), 1491 (vs), 1440 (vs), 1397 (vs), 1260 (vs), 1086 (vs), 786 (m). \textit{1H-NMR} (200 MHz, DMSO-\textit{d$_6$}, δ / ppm): 2.38 (3H, s, C7H$_3$), 2.58 (3H, s, C10H$_3$),
7.81 (1H, poorly resolved doublet, C4H), 8.01 (1H, poorly resolved triplet, C3H), 8.63 (1H, poorly resolved doublet, C2H), 8.65 and 8.75 (1H, s, N4H2+Hb), 8.97 (2H, s, N7H2), 10.85 (1H, s, N3H), 14.16 (1H, s, Se2). 13C-NMR (50.3 MHz, DMSO-d6, δ / ppm): 12.6 (C7+C10), 121.6 (C2+C4), 137.0 (C3), 149.9 (C6+C9), 153.7 (C1+C5), 175.5 (C8+C11).

**Complex 1.** Yield: 0.10 g (86 %); Anal. Calcd. for C11H13N7Se2Zn (FW: 466.58): C, 28.66; H, 3.52; N, 18.00 %. Found: C, 28.95; H, 3.51; N, 18.03 %. IR (KBr, cm⁻¹): 3411 (m), 3285 (m), 3157 (m), 1614 (m), 1482 (s), 1438 (vs), 1405 (s), 1285 (m), 1167 (m), 1024 (m), 714 (w). 1H-NMR (200 MHz, DMSO-d6, δ / ppm): 2.46 (6H, s, C7H3+C10H3), 6.82 (4H, s, N4H2+N7H2), 7.61 (2H, poorly resolved doublet, C2H+C4H), 7.92 (1H, poorly resolved triplet, C3H). 13C-NMR (50.3 MHz, DMSO-d6, δ / ppm): 17.4 (C7+C10), 124.1 (C2+C4), 138.9 (C3), 153.6 (C6+C9), 155.8 (C1+C5), 173.0 (C8+C11). Conductivity (ΛM / Ω⁻¹cm² mol⁻¹ (1×10⁻³ mol dm⁻³ in DMF)): 0.73.

**Complex 2.** Yield 0.07 g (55 %); Anal. Calcd. for C11H13N7Se2Cd (FW: 513.60): C, 25.72; H, 2.55; N, 19.09 %. Found: C, 25.66; H, 2.54; N, 18.53 %. IR (KBr, cm⁻¹): 3476 (m), 3285 (m), 3162 (w), 1607 (m), 1495 (s), 1435 (m), 1407 (s), 1170 (m), 1019 (w). 1H-NMR (200 MHz, DMSO-d6, δ / ppm): 2.42 (6H, s, C7H3+C10H3), 6.93 (4H, s, N4H2+N7H2), 7.80 (2H, d, 3J = 7.9 Hz, C2H+C4H), 8.12 (1H, t, 3J = 7.9 Hz, C3H). 13C-NMR (50.3 MHz, DMSO-d6, δ / ppm): 14.6 (C7+C10), 121.9 (C2+C4), 145.4 (C6+C9), 150.5 (C1+C5), 174.6 (C8+C11). Conductivity (ΛM / Ω⁻¹cm² mol⁻¹ (1×10⁻³ mol dm⁻³ in DMF)): 1.17.

**Complex 3.** Yield 0.26 g (84 %); Anal. Calcd. for C11H11N7SeNi (FW: 378.91): C, 34.87; H, 2.93; N, 25.88 %. Found: C, 34.64; H, 2.86; N, 25.47 %. IR (KBr, cm⁻¹): 3430 (m), 3315 (m), 3153 (s), 2176 (vs), 1648 (s), 1595 (w), 1535 (s), 1498 (s), 1444 (m), 1407 (m), 1370 (m), 1329 (m), 1289 (m), 1223 (w), 1172 (m), 1107 (m), 1076 (m), 1045 (m), 797 (m), 766 (w), 712 (m). 1H-NMR (200 MHz, DMSO-d6, δ / ppm): 2.34 (3H, s, C10H3), 2.51 (3H, s, C7H3), 7.87 (2H, s, N4H2), 7.98 (1H, poorly resolved doublet, C2H), 8.18 (1H, poorly resolved doublet, C4H), 8.41 (1H, poorly resolved triplet, C3H). Conductivity (ΛM / Ω⁻¹cm² mol⁻¹ (1×10⁻³ mol dm⁻³ in DMF)): 1.35.

**Free radical scavenging assay**

The total antioxidant activity assay using ABTS cation radicals was performed according to the previously published procedure with some modifications. Briefly, ABTS was dissolved in water to a concentration of 7 mM and its cation radicals were produced in a reaction with 2.45 mM potassium persulphate. The resulting mixture was kept in the dark at room temperature for 12–16 h before use. Prior to the assay, the solution was diluted with miliQ water (about 1:79, v/v) to give an absorbance of 0.70±0.02 at 734 nm. After the addition of 2 mL of diluted ABTS cation radical solution to 0.2 mL of a fixed concentration of the compounds, the absorbance at 734 nm was recorded exactly 30 min after the initial mixing. Results are presented as mean ± standard deviation. A minimum of three independent experiments were performed. The log sigmoid dose–response curves of free radical scavenging activity of tested compounds were generated using the Origin 7.0 software package (Microcal Software Inc., Northampton, MA, USA). The IC₅₀ values were determined by post hoc regression analysis of the linear segments of the sigmoid dose response curves. Calibration curves of the standards and samples were considered as linear if R² > 0.98.

**Animals**

This study was run in accordance to the statements of European Union regarding handling of experimental animals (86/609/EEC), and approved by the Ethical Committee for Labo-
Acute oral toxicity study

Acute oral toxicity test was run strictly in accordance with OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects: Test No. 423: Acute Oral toxicity – Acute Toxic Class Method. The principle of the test is that, based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of a test substance. The substances were tested using a stepwise procedure. In each step, three female mice were used since they are generally slightly more sensitive. The absence or presence of compound-related mortality of the animals dosed at one step determined the next step, i.e., no further testing is needed, dosing of three additional animals with the same dose, or dosing of three additional animals at the next higher or the next lower dose. The animals, which were 8–10 weeks old and weighed 22–30 g, were fasted for 4 h before the experiment and divided into experimental and control groups. Test samples were dissolved first in DMSO, and distilled water was added to obtain the final concentration of the tested compounds (the final concentration of DMSO was 20 %). The control group was treated with 20 % DMSO. A single dose of 300 and 2000 mg kg\(^{-1}\) b.wt. of Ni (II), Cd(II) and Zn(II) complexes was administered intragastrically (i.g.) using a metal tube for gavage. Fixed volume of 0.5 mL was administrated per mouse. Food was returned to the animals 3 h after dosing. Individual weights were determined at the start of the fasting time (day 0), immediately before the test substance was administered (day 0) and on the test days 1, 2, 4, 7, and 14. The animals were observed for clinical signs of toxicity while handled before and after fasting, during the first 30 min after dosing, at least two more times within 4–4.5 h after dosing, and daily thereafter. Observations for abnormal behavioural signs, somnolence, dizziness, restlessness, neurological signs, respiratory distress or mortality were conducted twice daily. At the end of the test (day 14), the mice were sacrificed by cervical dislocation, and gross pathological changes in the main organs (brain, liver, kidney, spleen, gastric and intestinal mucosa) were evaluated.

RESULTS AND DISCUSSION

The biological activities of semicarbazones and thiosemicarbazones were reported to increase upon coordination with the metal ions. The metal complex could be more active than the free ligand, and could exhibit biological activities that are not shown by the free ligand. In addition, some adverse effects may decrease upon complexation. Moreover, coordination may lead to significant reduction of drug-resistance. Therefore, studies on novel metal-based compounds with therapeutic potential have become an area of intense investigation in biomedicine and inorganic chemistry. In the present study, the antioxidative potential and acute toxicity level of three synthesised selenosemicarbazone metal complexes 1–3 (Scheme 1) were investigated.

There are various antioxidant activity assays, each having their specific target within the matrix and all of them with advantages and disadvantages. The
ABTS assay used in this study represents one of the most important and widely used assays. This colorimetric test provides determination of the antioxidant capacity of natural and synthetic compounds since the ABTS radical decolorizes in the presence of antioxidants. The free radical scavenging capacity (FRSC in %) of the ligand H$_2$dapsesc, the complexes 1–3 and metal salts used in synthesis, as well as the ascorbic acid as positive control and DMSO as the vehicle control is expressed as 50 % inhibitory concentration ($IC_{50}$ in $\mu$M, Table I), defined as the concentration of the tested compound leading to a 50 % reduction of the free radical concentration. The $IC_{50}$ values for the tested compounds were calculated from log sigmoidal dose response inhibition curves by post hoc linear regression analysis. These results clearly indicated that the complexes 1–3 exhibited an excellent ABTS radical cation-scavenging effect, comparable to that of a reference antioxidant, Vitamin C. The antioxidative capacity of the ligand was lower by an order of magnitude compared to the activity of the complexes. It is to be noted that no significant radical scavenging activities were observed in the experiment carried out under the same experimental conditions with the metal salts used in the syntheses of the complexes, even up to the concentration of 1.0 mM. The $IC_{50}$ values (Table I) indicated that the ligand and the complexes showed antioxidant activity in the following order: 2 > 1 > 3 > H$_2$dapsesc. It can be assumed that coordination of the ligand in anionic form promotes the ligand-centered reduction of the CH$_3$C=N double bond in the complexes, which is reflected in the higher antioxidative potential of the complexes compared to the free ligand, as was registered for related Ga(III) complexes with thiosemicarbazones.

TABLE I. $IC_{50}$ values (in $\mu$M) calculated from ABTS radical cation scavenging assay of the tested compounds and the standard (vitamin C)

<table>
<thead>
<tr>
<th>Compound</th>
<th>$IC_{50}$ / $\mu$M</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$dapsesc</td>
<td>557±54</td>
</tr>
<tr>
<td>[Zn(dapsesc)] (1)</td>
<td>29.51±1.50</td>
</tr>
<tr>
<td>[Cd(dapsesc)] (2)</td>
<td>21.62±0.83</td>
</tr>
<tr>
<td>[Ni(hcn)] (3)</td>
<td>35.59±5.30</td>
</tr>
<tr>
<td>Zn(CH$_3$COO)$_2$·2H$_2$O</td>
<td>&gt; 1000</td>
</tr>
<tr>
<td>Cd(CH$_3$COO)$_2$·2H$_2$O</td>
<td>&gt; 1000</td>
</tr>
<tr>
<td>Ni(CH$_3$COO)$_2$·4H$_2$O</td>
<td>&gt; 1000</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>21.35±2.75</td>
</tr>
</tbody>
</table>

It is known that selenium represents one of the most important micro-nutrients in human diet regarding antioxidant activity. It does not act directly on free radicals but is an indispensable part of most antioxidant enzymes (metallo-enzymes, glutathione peroxidase) that would have no effect without it. The mode of anticancer action of Se is not fully understood but several mechanisms including antioxidant protection by selenoenzymes have been proposed. Furthermore, zinc is a bio-element that is important in the prevention of free radicals
formation. It plays a role as an inhibitor of NADPH oxidases, which catalyze the production of the superoxide radical anion from oxygen by using NADPH as an electron donor. Zinc is present in superoxide dismutase, an important antioxidant enzyme that converts the superoxide radical anion into hydrogen peroxide. Finally, zinc induces the production of metallothionein that is a scavenger of the hydroxyl radical, and also competes with copper for binding to the cell wall, thus decreasing the production of hydroxyl radicals. Taken together, the present preliminary in vitro results and these known properties of selenium and zinc allow the proposal that these metal complexes are worthy of further investigation under in vivo conditions.

However, the toxicity and carcinogenicity of metal ions should not be neglected. The primary route for their toxicity is depletion of glutathione and bonding to sulfhydryl groups of proteins. In addition, the unregulated intake of dietary or pharmacological selenium, mainly in the form of the inorganic Se compound, sodium selenite, could potentially expose the body tissues to toxic levels of Se with the subsequent negative consequences on DNA integrity. Due to a broad interest for the beneficial effects of Se on human health and cancer prevention and therapy, studies investigating the negative effects such as toxicity from high Se intake are also highly required. Therefore, a toxicological study of the investigated selenosemicarbazone metal complexes was conducted as one of the most important steps required for further studies of biological activity. The acute toxicity evaluation was based on an established protocol, internationally recognized as a reference standard tool for chemical tests. The OECD 420 guideline – Fixed-Dose Procedure for Assessing Oral Acute Toxicity was followed. Acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance. According to globally harmonized system of classification and labelling of chemicals (GSH), substances are assigned to one of the five toxicity categories based on LD₅₀ (oral and dermal) or LC₅₀ (inhalation). For the evaluation of acute oral toxicity, a single dose (300 and 2000 mg kg⁻¹) was orally administered to female mice. The doses used were based on the previous toxicological data on the structurally related substance, N-4-[(E)-((aminothioxomethyl)hydrazono)methyl][phenyl]acetamide (thioacetazone), which was helpful in the selection of the most appropriate initial dose. Namely, the median lethal dose (LD₅₀) for thioacetazone was determined as 950 mg kg⁻¹ after oral administration in mouse. Thus a starting dose 300 mg kg⁻¹ was employed in the present study. The results are presented in Table II.

At the dose level of 300 mg kg⁻¹ body weight, no mortality was observed, but some signs and unusual symptoms during the first 4 h of the acute toxicity protocol were observed, such as exciting behavioural (agitation, touch response), inhibitory (sleepiness) and other characteristics (piloerection). It was observed that the compounds did not cause any gross behavioural alterations, such as
convulsion, dizziness or respiratory distress. No clinical signs of toxicity were observed in the group that received \([\text{Ni(hcn)}]\) in the lower dose. After that, the toxicity level of the \(\text{Ni(II)}\), \(\text{Cd(II)}\) and \(\text{Zn(II)}\) selenosemicarbazone complexes were evaluated at the maximum dose of 2000 mg kg\(^{-1}\) administered orally to the 4 h-fasted mice. At the higher dose, toxic symptoms, such as mild agitation, touch response in one animal and sleepiness in the \([\text{Cd(dapsesc)}]\) group, ataxia and somnolence with a fatal effect in \([\text{Ni(hcn)}]\) group, were observed. During the study period of two weeks, no death occurred in the animals treated with the \(\text{Cd(II)}\) and \(\text{Zn(II)}\) selenosemicarbazone complexes, which indicates that the lethal dose of the compounds is above 2000 mg kg\(^{-1}\) body weight in mice and that the compounds could be considered to be less harmful at this dose. No test substance-related effects on body weight occurred. The mean body weights of the test mice were similar to those of the control group throughout the study (data not shown). Sporadic body weight losses of up to 5 % were not considered test substance-related, because the decreases were only over a one- or two-day period and did not occur the day after dosing. No gross lesions were present in the mice at necropsy. In addition, macroscopic examination revealed that the organs taken presented aspects of colour, size and texture, showing no difference from the control group.

**TABLE II. Parameters of acute toxicity observed in adult female mice, treated with different oral doses of Ni(II), Cd(II) and Zn(II) selenosemicarbazone complexes; –, no effect; +, mild effect; ++, moderate effect; +++ major effect**

<table>
<thead>
<tr>
<th>Clinical sign</th>
<th>Compound/Dose, mg kg(^{-1})</th>
<th>Control (20 % DMSO)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>([\text{Ni(hcn)}])</td>
<td>([\text{Cd(dapsesc)}])</td>
</tr>
<tr>
<td>Agitation</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Convulsion</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ataxia</td>
<td>–</td>
<td>+++</td>
</tr>
<tr>
<td>Touch response</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Piloerection</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sleepiness</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Somnolence</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Respiratory distress</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mortality</td>
<td>0/6</td>
<td>6/6</td>
</tr>
</tbody>
</table>

The absence of mortality at concentrations as high as 2000 mg kg\(^{-1}\) b.wt. of the \(\text{Cd(II)}\) and \(\text{Zn(II)}\) complexes did not allow the calculation of the median lethal dose (\(LD_{50}\)) value. Hence, the \(\text{Cd(II)}\) and \(\text{Zn(II)}\) complexes investigated in this study belong to the so-called category 5 or unclassified according to GSH classification.\(^{31}\) Therefore, these compounds show low acute toxicological risk. The absence of gross behavioural alteration is also another indication of lack of toxicity of the compounds. Moreover, it is important to emphasize that a safe
therapeutic index or therapeutic ratio could be expected. A higher therapeutic index is preferable to a lower one, and means that much higher doses of these compounds are required to reach the lethal/toxic threshold than the dose which will lead to a therapeutic effect.

CONCLUSIONS

The investigated compounds showed high in vitro antioxidative potential. Accordingly, they are good candidates for the prevention or treatment of a broad range of pathological conditions mediated by uncontrolled oxidative processes. They also exhibited low toxic effects as required for further in vivo therapeutic studies. The obtained results invoke further preclinical studies that should show their efficacy in different oxidative stress related disorders. In addition, long-term toxicological studies with repeated doses are necessary for the final safety evaluation.

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