Synergy of experimental and computational chemistry: structure and biological activity of Zn(II) hydrazone complexes†

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In this paper, three different Zn(II) complexes with (E)-2-(2-(1-(6-bromopyridin-2-yl)ethylidene)hydrazinyl)-N,N,N-trimethyl-2-oxoethan-1-aminium chloride (HLCl) have been synthesized and characterized by single crystal X-ray diffraction, elemental analysis, IR and NMR spectroscopy. All complexes are mononuclear, with the ligand (L) coordinated in a deprotonated formally neutral zwitterionic form via NNO donor set atoms. Complex 1 forms an octahedral geometry with the composition [ZnL3][BF4]2, while complexes 2 [ZnL(NCO)2] and 3 [ZnL(N3)2] form penta-coordinated geometry. Density functional theory (DFT) calculations were performed to enhance our understanding of the structures of the synthesized complexes and the cytotoxic activity of the complexes was tested against five human cancer cell lines (HeLa, A549, MDA-MB-231, K562, LS 174T) and normal human fibroblasts MRC-5. Additionally, antibacterial and antifungal activity of these complexes was tested against a panel of Gram-negative and Gram-positive bacteria, two fungal strains, and a yeast strain. It is noteworthy that all three complexes show selective antifungal activity comparable to that of amphotericin B. Molecular docking analysis predicted that geranylgeranyl pyrophosphate synthase, an enzyme essential for sterol biosynthesis, is the most likely target for inhibition by the tested complexes.

Hydrazone ligands, derived from the condensation of hydrazone and carbonyl compounds, exhibit a wide range of biological activities, including antimicrobial, anticancer, anti-inflammatory, and enzyme inhibition properties. The modularity and tunability of hydrazone scaffolds allow for the facile modification of their physicochemical properties and pharmacological profiles, making them attractive candidates for drug design and development. In particular, the coordination of hydrazone ligands to metal ions, such as zinc, enhances their stability, bioavailability, and target specificity, leading to improved therapeutic efficacy and reduced toxicity. Moreover, hydrazone ligands can be easily modified by introducing different substituents at the hydrazine or carbonyl unit, which can affect the electronic and steric properties of the ligands and the complexes.

MicroRNAs (miRNAs) are small RNA molecules that regulate gene expression by incompletely complementary binding to their targets, thus inducing translational repression of mRNA or degradation. The main characteristic of microRNA molecules is reflected in their possibility to silence various numbers of messenger RNAs, thus repressing translation and acting as tumor suppressors, oncogenes, or even both, depending on cellular physiological and molecular context. Treating cells with complexes may result in transcriptional changes and...
affect the expression of genes and microRNAs, thus modulating downstream signaling pathways. Matrix metalloproteinase 9 (MMP9) and vascular endothelial growth factor (VEGFA) are highly investigated molecules with oncogenic potential related to cervical cancer formation and progression.24,25 Understanding the interactions of zinc complexes with biomolecules is crucial for elucidating their therapeutic potential in various diseases. Computational chemistry has emerged as a valuable tool for unraveling the structural intricacies and elucidating the reactivity patterns of metal complexes. Computational methods, such as density functional theory (DFT), provide invaluable insights into the electronic structures and bonding nature of zinc complexes. A detailed understanding of their binding mechanisms with DNA and different proteins is essential for optimizing their efficacy and selectivity. Molecular docking, a computational technique, allows for exploring the binding modes and affinity between small molecules and biomolecular targets, providing insights into their potential biological activities.26,27

Complexes with ligands based on 2-acetylpyridine and its derivatives have attracted attention in medicinal chemistry due to their diverse biological activities. These ligands are versatile and often coordinate with metal ions to form stable complexes that exhibit a wide range of biological activities, including antimicrobial12–14,15 anticancer16 and catalytic properties.17 Given the encouraging properties of complexes with hydrazone ligands derived from 2-acetylpyridine and its derivatives, and as an extension of the previous studies carried out by our group, a ligand with Girard’s T reagent and a 2-acetylpyridine derivative was selected for the subsequent synthesis of complexes with zinc ions and evaluation of their activity.

In this paper, we report the synthesis, characterization, and biological assessment of three new zinc complexes with hydrazone ligand derived from 2-acetyl-6-bromopyridine and Girard’s T reagent. The complexes have different pseudohalogenes (N₃− and OCN−) in their structure and coordination geometries (distorted trigonal bipyramidal and octahedral). Computational studies were performed on the complexes using DFT and molecular docking methods to investigate their structural and electronic properties, and their interactions with proteins. All complexes were tested for antimicrobial activity against eight strains of bacteria, two strains of fungi, and one yeast strain, and their cytotoxic activity was determined against five cancer cell lines and one normal cell line. The mechanisms of anticancer effects of complexes were further examined in cervical adenocarcinoma HeLa cells, while their potential antioxidant effects were investigated in normal fibroblasts.

Through this interdisciplinary study, we aim to gain insight into the structure and biological activity of zinc complexes with hydrazone ligands, to explore their potential as novel cytotoxic and antimicrobial agents, to increase our knowledge of the anticancer mechanism of Zn(n) complexes, and ultimately to provide a thoughtful contribution to the design of new coordination compounds with tailored properties for various applications.

### Results

#### General

The condensation of 2-acetyl-6-bromopyridine with Girard’s T reagent, (carboxymethyl)trimethylammonium chloride hydrazide, in methanol produced the ligand HCl (E)-2-(1-(6-bromopyridin-2-yl)ethylidene)hydrazinyl)-N,N,N-trimethyl-2-oxoethan-1-aminium chloride (Scheme 1). Complex 1, an octahedral complex containing two molecules of the coordinated ligand [ZnL₂](BF₄)₂, was obtained when the ligand HCl reacted with Zn(BF₄)₂·6H₂O and NaN₃ (1 : 1 : 4). In complex 2, with the composition [ZnL(NCO)]₃, Zn(n) is pentacoordinated, with a distorted trigonal bipyramidal geometry (a similar geometry is observed in complex 3). The interactions between Zn (BF₄)₂·6H₂O with ligand HCl and NaOCN in water/methanol solution (1 : 2 v/v) produced complex 2.

Based on previously published work,36 we tried increasing the amount of azide after the synthesis of complex 1 to see if the same product was produced, which would be helpful for directed synthesis in the future.

By reacting HCl with Zn(BF₄)₂·6H₂O and NaN₃ in large excess (1 : 1 : 12) in the same solvent combination as in the previous reaction (reaction conditions used for the synthesis of complex 1), the mononuclear Zn(n) complex 3, with the composition [ZnL(N₃)]₃, was formed (Scheme 1). In short, increasing the amount of azide leads to forming 3, where one deprotonated ligand and two azide ligands are coordinated to Zn(n).

The stability of all three zinc complexes at a concentration of 10⁻³ M in water, methanol and DMSO was investigated by monitoring the change in absorption spectrum over 24 h at room temperature using UV-Vis spectrometry. The comparison of the spectra recorded in freshly prepared solutions and after

![Scheme 1](image.png)
The coordination of the ligand through the imine nitrogen, oxygen from the carbonyl group, and pyridine nitrogen was verified by IR spectroscopy. The IR spectrum of the ligand shows a broad band at 3377.4 cm⁻¹, which is the stretching vibrational mode of the N–H group. This band disappeared in the spectra of three Zn(II) complexes 1–3. An extremely strong band at 1703.4 cm⁻¹, corresponding to the carbonyl group’s C=O stretching vibration, is also visible in the ligand spectrum. This peak is absent from the spectra of all three complexes; instead, strong peaks originating from the C=O stretching vibration can be seen at 1026.3 cm⁻¹ (complex 1), 1027.8 cm⁻¹ (complex 2) and 1120.6 cm⁻¹ (complex 3). These factors point to the carbonyl group’s electron pair delocalizing toward oxygen, subsequently promoting the ligand’s coordination through the negatively charged oxygen atom. A band at 2209 cm⁻¹ observed in the IR spectrum of 2 corresponds to the cyanate coordination, while the presence of the N₃⁻ group in the complex structure is shown by the noticeable band that appears at 2060.4 cm⁻¹ in the infrared spectrum of complex 3. The IR spectra of the ligand and the Zn(II) complexes are given in the ESI (Fig. S4–S7).

**Crystal structures of Zn(II) complexes**

**Crystal structure of complex 1.** Complex 1, [Zn(L)]²⁺, crystallized in orthorhombic Pbcn space group as one independent complex cation [ZnL₂]²⁺ and two BF₄⁻ anions. The structure of [ZnL₂]²⁺ with the atomic numbering scheme is shown in Fig. 1. The bond lengths and valence angles of complex 1 are listed in Table 1. Two ligand molecules [ZnL]²⁺ coordinate to the Zn(II) ion in a meridional fashion, forming a distorted centrosymmetric octahedral structure by chelation through two NNO donor atom sets. Each ligand coordinates to Zn(II) through 2-bromopyridine, imine nitrogen, and enolate oxygen atoms. The tridentate coordination of each ligand implies the formation of two fused five-membered chelate rings Zn–N–C–C–N and Zn–N–N–C–O. The pair of the five-membered chelate rings Zn1–N1–C5–C6–N2 and Zn1–N2–N3–C8–O1 is nearly
co-planar, as indicated by the dihedral angle of 9.27°. The chelation planes comprising the atoms N, N, O, and Zn(II) are almost perpendicular with the dihedral angle of 84.9°. The Zn–NAr 2.272(2) Å, Zn–Nimine 2.047(2) Å, and Zn–O 2.114(2) Å bond lengths are comparable to those observed for one ligand L in Zn(n) complexes 2 and 3.

**Crystal structures of complexes 2 and 3.** Complexes 2 ([ZnL(NCO)₂]) and 3 ([ZnL(N₃)₂]) both crystallize as independent molecules in the asymmetric unit of the triclinic P₁ space group. The molecular structures with the atomic numbering scheme are shown in Fig. 2 and 3. The bond lengths and valence angles are listed in Table 1.

Two nitrogen atoms (N1 and N2) and one oxygen atom (O1) of the tridentate ligand L are coordinated to the Zn(n) ion in both structures. Two N₃⁻ or two NCO⁻ ligands fulfill the coordination sphere to complete the distorted trigonal bipyramidal coordination.

The tridentate NNO coordination of L to Zn(n) ion generates two five-membered chelate rings (Zn–N–C–C–N and Zn–N–N–C–O) which are almost co-planar, as indicated by the dihedral angle between chelate ring planes of 3.4° in 3 and 1.6° in 2, respectively.

This is less than the corresponding Ni(n) complex, which has the same chelate rings of tridentate donor ligands and three coordination azide anions in the crystal structure. The Zn–N₄ (2.332(2) and 2.319(4) Å), Zn–Nimine (2.046(3) and 2.057 (4) Å) and Zn–O (2.161(2) and 2.133(4) Å) are close in both complexes. Zn–Nazido (1.971(3) and 1.978(3) Å) and Zn–Ncyanato (1.940(5) and 1.954(5) Å) are almost the same as in similar complexes. The azido ligands in 3 are nearly linear (angle N–N–N is 177°) and slightly asymmetric (N5–N6 = 1.183(4) Å and N6–N7 = 1.144(4) Å; N8–N9 = 1.187(4) Å and N9–N10 = 1.158(4) Å) indicating that the shorter bonds are remote from the metal center. A similar situation can be found in complex 2: the cyanate ligands perform asymmetry (e.g. N5–C13 = 1.153(7) Å and C13–O2 = 1.196(7) Å). The N–N–Zn bond angles in 3 are 121.5(3)° and 123.2(2)° showing bent coordination of the anionic terminals. Such bent coordination is less pronounced in 2 (e.g. 153.2(4)°).

**DFT studies.**

We conducted density functional theory (DFT) calculations to enhance our understanding of the structures of synthesized complexes. Starting from the X-ray-determined structures of complexes 1–3, we explored the thermodynamics of potential reactions in solution. Free energy changes (ΔrG at 298 K) are summarized in Table 2. Geometry optimization, normal mode frequencies and electronic energy calculations were conducted using ZORA-BP86-D4/TZP-COSMO46 and ZORA-CAM-B3LYP/TZP-COSMO46 levels of theory, respectively. Results confirm the stability of the complexes in various solvents and suggest that cyanate ligand binds via nitrogen in complex 2. Ligand substitution by N₃⁻ or OCN⁻ is having

![Fig. 2 ORTEP representation of the [ZnL(NCO)₂] complex. Thermal ellipsoids are drawn at the 30% probability level.](image)

![Fig. 3 ORTEP representation of the [ZnL(N₃)₂] complex. Thermal ellipsoids are drawn at the 30% probability level.](image)

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Δ̂G (298 K)</th>
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<tbody>
<tr>
<td>[ZnL₂⁺]²⁺ + 2H₂O = 2[ZnL(H₂O)]⁺ + L &amp; ΔG° + 22.2a</td>
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<tr>
<td>[ZnL(N₃)₂] + 2H₂O = 2[ZnL(H₂O)]⁺ + 2N₃⁻ &amp; ΔG° + 14.4a</td>
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<tr>
<td>[ZnL(NCO)₂] + 2H₂O = 2[ZnL(H₂O)]⁺ + 2OCN⁻ &amp; ΔG° + 16.3a</td>
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<tr>
<td>[ZnL₂⁺]²⁺ + CH₃OH = 2[ZnL(CH₃OH)]²⁺ + L &amp; ΔG° + 24.4b</td>
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<tr>
<td>[ZnL(N₃)₂] + 2CH₃OH = 2[ZnL(CH₃OH)]²⁺ + 2N₃⁻ &amp; ΔG° + 19.8b</td>
<td></td>
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<tr>
<td>[ZnL(NCO)₂] + 2CH₃OH = 2[ZnL(CH₃OH)]²⁺ + 2OCN⁻ &amp; ΔG° + 21.3b</td>
<td></td>
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<tr>
<td>[ZnL₂⁺]²⁺ + 2DMSO = 2[ZnL(DMSO)]²⁺ + L &amp; ΔG° + 24.4c</td>
<td></td>
</tr>
<tr>
<td>[ZnL(N₃)₂] + 2DMSO = 2[ZnL(DMSO)]²⁺ + 2N₃⁻ &amp; ΔG° + 17.3c</td>
<td></td>
</tr>
<tr>
<td>[ZnL(NCO)₂] + 2DMSO = 2[ZnL(DMSO)]²⁺ + 2OCN⁻ &amp; ΔG° + 19.8c</td>
<td></td>
</tr>
<tr>
<td>[ZnL(NCO)₂] = [ZnL(OCN)] &amp; ΔG° + 18.5c</td>
<td></td>
</tr>
<tr>
<td>[ZnL₂⁺]²⁺ + 2OCN⁻ = [ZnL(NCO)₂] + L &amp; ΔG° + 4.9d</td>
<td></td>
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<tr>
<td>[ZnL₂⁺]²⁺ + 2N₃⁻ = [ZnL(N₃)₂] + L &amp; ΔG° + 3.4d</td>
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lower $\Delta G$ than in the case of substitution by solvent molecules, necessitating an excess of azide or cyanate for synthesis of 2 and 3.

Electrostatic potential surfaces of $\text{HL}^+$, $\text{L}$, and complexes 1–3 are presented in Fig. 4 using “divergent colormap”. The charge distributions are illustrated in red/blue, indicating negatively/positively charged areas. Positively charged regions are mainly within the quaternary ammonium group. In complexes 2 and 3, negatively charged areas are concentrated around the monodentate anionic ligands and bromine atom of the $\text{L}$. In $[\text{ZnL}_2]^{2+}$, the least positive parts are around the bromine atoms of the coordinated $\text{L}$. Fig. 4 clearly shows the NNO negatively charged region in deprotonated $\text{L}$ that will coordinate to the Zn(II) ion.

The highest occupied (HOMO) and lowest unoccupied (LUMO) molecular orbitals of investigated molecules are shown in Fig. 5 and 6. The frontier orbitals of all three complexes are localized on the tridentate ligand. Apart from the slight delocalization of HOMO of 3 on azide monodentate ligands, frontier orbitals resemble the orbitals of the tridentate ligand itself.

Further insight into the electronic structure of studied complexes is obtained from conceptual DFT. The conceptual DFT descriptors of studied complexes 1–3 and free $\text{HL}^+$ are evaluated at CAM-B3LYP/TZP-COSMO(water)/ZORA-BP86-D4/TZP-COSMO(water) level of theory employing the finite difference linearization (FDL) approach. Herein, we focus on four key global molecular descriptors: (i) the electronic chemical potential ($\mu$), which reflects the energy required to add or remove an electron from the system and provides insights into the tendency of a system to undergo electronic changes; (ii) the molecular hardness ($\eta$) that quantifies the resistance of a molecule to electron transfer; in orbital approximation HOMO–LUMO gap is attributed to the molecular hardness; (iii) the molecular softness ($S$) that characterizes the responsiveness of a molecule to external perturbations, i.e. high softness is associated with high polarizability; and (iv) the electrophilicity index ($\omega$) that measures the tendency of a molecule to accept electrons. These descriptors are intrinsic molecular properties directly derived from DFT calculations and are given in Table 3. $\text{HL}^+$, being a cation, shows low $\mu$ and relatively high $\omega$, i.e., a tendency to gain electrons. $\text{HL}^+$ is, at the same time, hard. Coordination of tridentate ligand to Zn(II) changes molecular properties. Complexes 1–3 are softer, i.e., more polarizable than free ligand. Complexes 2 and 3 are similar, showing

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<tr>
<th></th>
<th>$\mu$</th>
<th>$\eta$</th>
<th>$S$</th>
<th>$\omega$</th>
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</thead>
<tbody>
<tr>
<td>$\text{HL}^+$</td>
<td>$-0.170$</td>
<td>$0.159$</td>
<td>$6.282$</td>
<td>$0.090$</td>
</tr>
<tr>
<td>$[\text{ZnL}_2]^{2+}$</td>
<td>$-0.158$</td>
<td>$0.148$</td>
<td>$6.757$</td>
<td>$0.084$</td>
</tr>
<tr>
<td>$[\text{ZnL(NCO)}_2]$</td>
<td>$-0.158$</td>
<td>$0.129$</td>
<td>$7.731$</td>
<td>$0.096$</td>
</tr>
<tr>
<td>$[\text{ZnL(N3)}_2]$</td>
<td>$-0.165$</td>
<td>$0.138$</td>
<td>$7.232$</td>
<td>$0.098$</td>
</tr>
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high softness, high values of $\omega$, and relatively low $\mu$. The dual descriptor of the Fukui function is depicted in Fig. S16.†

A detailed picture of bonding in complexes 1–3 is gained by the extended transition state Energy Decomposition Analysis (EDA).51–53 In this approach, chemical bonding between fragments is analyzed stepwise, decomposing the interaction energy, $E_{\text{int}}$, into chemically meaningful components. The first component is the quasi-classical electrostatic interaction between the fragments ($E_{\text{elst}}$). The second component is the repulsive Pauli interaction ($E_{\text{Pauli}}$). The third component is the orbital stabilizing contribution ($E_{\text{orb}}$). Finally, dispersion correction ($E_{\text{disp}}$) arises if Grimme’s type dispersion energy correction (D4) is included. Pauli and orbital deformation densities are depicted in Fig. S17 in ESL.†

Results of EDA analysis at ZORA-BP86-D4/TZP level of theory for bonding [ZnL]$^{2+}$–L, [ZnL]$^{2+}$⋯(NCO)$_2$–, [ZnL]$^{2+}$⋯(N$_3$)$_2$– are given in Table 4. The situation [ZnL]$^{2+}$⋯(OCN)$_2$– is also considered to understand the preferential bonding via the N atom in the cyanate complex.

Electrostatic interactions are the primary stabilizing terms in the structures, especially for charged fragments like [ZnL]$^{2+}$⋯(NCO)$_2$–, [ZnL]$^{2+}$⋯(N$_3$)$_2$–, [ZnL]$^{2+}$⋯(OCN)$_2$–, where it accounts for approximately 70–75–85% of the stabilization. $E_{\text{elst}}$ contributes around 25% to the stabilization in these three cases and around 35% for [ZnL]$^{2+}$…L. To clarify the contribution of sole covalency in orbital term, NOCV analysis54,55 has been performed. The most crucial density deformation channels related to the $\sigma$-covalency and $\pi$-covalency are depicted in Fig. 7, and quantified. Their energy contribution is given in Table 4 ($E_{\sigma1}$ and $E_{\sigma2}$).

Cytotoxic activities of Zn(II) complexes

Cytotoxic effects of three novel Zn(II) complexes and their precursors were tested against five human cancer cell lines and normal human fibroblasts (Table 5). Among examined complexes, Zn(II) complex 1 exerted the strongest cytotoxic activity on chronic myelogenous leukemia K562 cells with an IC$_{50}$ value of 93.62 $\mu$M. This complex showed the cytotoxic effect on cervical adenocarcinoma HeLa cells with an IC$_{50}$ value of 136.47 $\mu$M. Compared to activity on K562 and HeLa cells, complex 1 had lower cytotoxicity against breast adenocarcinoma MDA-MB-231 and colorectal adenocarcinoma LS 174T cells. The lowest cytotoxic activity was observed on lung carcinoma A549 cells. Zn(II) complex 2 showed the strongest cytotoxic effect against K562 and HeLa cells. The lower intensity of cytotoxic activity of complex 2 was observed

### Table 4 Energy decomposition analysis of $[\text{ZnL}]^{2+}$⋯X (X = L, (NCO)$_2$, (N$_3$)$_2$, (OCN)$_2$) at ZORA-BP86-D4/TZP level of theory. Energy components are given in kcal mol$^{-1}$ relative to the chosen fragments; $\Delta q$ is the Hirshfeld charge transferred between fragments

<table>
<thead>
<tr>
<th>Complex</th>
<th>$E_{\text{Pauli}}$</th>
<th>$E_{\text{elst}}$</th>
<th>$E_{\text{orb}}$</th>
<th>$E_{e1}$</th>
<th>$E_{e2}$</th>
<th>$E_{\text{disp}}$</th>
<th>$E_{\text{dist}}$</th>
<th>$\Delta q$</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ZnL]$^{2+}$⋯L</td>
<td>134.6</td>
<td>-160.4</td>
<td>-105.8</td>
<td>-43.9</td>
<td>-15.3</td>
<td>-21.9</td>
<td>-153.6</td>
<td>0.55</td>
</tr>
<tr>
<td>[ZnL]$^{2+}$⋯(NCO)$_2$</td>
<td>195.6</td>
<td>-429.3</td>
<td>-166.3</td>
<td>-73.5</td>
<td>-25.5</td>
<td>-12.8</td>
<td>-412.9</td>
<td>0.57</td>
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<tr>
<td>[ZnL]$^{2+}$⋯(N$_3$)$_2$</td>
<td>186.4</td>
<td>-423.4</td>
<td>-154.2</td>
<td>-65.3</td>
<td>-24.8</td>
<td>-13.8</td>
<td>-405.5</td>
<td>0.60</td>
</tr>
<tr>
<td>[ZnL]$^{2+}$⋯(OCN)$_2$</td>
<td>155.5</td>
<td>-392.5</td>
<td>-122.9</td>
<td>-47.3</td>
<td>-18.0</td>
<td>-14.0</td>
<td>-373.9</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Fig. 7 Most important covalent deformation density channels from NOCV analysis of $[\text{ZnL}]^{2+}$⋯X (X = L, (NCO)$_2$, (N$_3$)$_2$, (OCN)$_2$) (isosurfaces 0.005 au), $e1$ (left) and $e2$ (right) calculated at ZORA-BP86-D4/TZP level of theory. Yellow/blue color represent electron outflow/inflow.
against MDA-MB-231 and LS 174T cells, while the lowest cytotoxicity was observed against A549 cells. Complex 3 had the lowest cytotoxic effects on examined cancer cell lines compared to complexes 1 and 2. This complex exhibited the highest cytotoxic activity against K562 cells. The lower cytotoxic effect was observed against HeLa cells, while very low cytotoxicity was found against LS 174T cells, MDA-MB-231, and A549 cells.

K562 cells were the most sensitive to the cytotoxic effects of each of the three examined Zn(n) complexes, being the most sensitive to the activity of complex 1. HeLa and LS 174T cells had similar sensitivity to the cytotoxic effects of complexes 1 and 2. MDA-MB-231 cells were the most sensitive to the cytotoxicity of complex 2, while A549 cells showed the lowest sensitivity to the cytotoxicity of three zinc complexes.

Each of the three complexes exerted higher cytotoxic activity against K562 and HeLa cancer cells than against normal human lung fibroblasts MRC-5. The best selectivity in the cytotoxic coefficient of 2.13 when comparing its cytotoxicity against normal MRC-5 cells and K562 cells. This complex also showed slightly higher cytotoxicity against K562 and HeLa cells than its ligand and precursor compounds.

The cytotoxic activities of three novel Zn(n) hydrazone complexes against HeLa cells were stronger when compared with the cytotoxic activity of Zn(n) complex with N,N,N-trimethyl-2-oxo-2-(2-1-(thiazol-2-yl)ethyl-ideny)hydrazinyl)ethan-1-aminium chloride against HeLa cells reported in our previous work with IC50 value of 289.10 µM.56 In contrast to results of present study, Araškov and colleagues showed high intensity of cytotoxic activity of Zn(n) complexes with thiazolyl-hydrazone ligands against HeLa and A549 cancer cell lines, in addition to HBL-100 and T-47D breast cancer cell lines and non-small cell lung cancer SW1573 cells with GI50 values in the range of 0.17 µM to 2.90 µM.57 Two Zn(n) complexes of aryl-hydrazone Schiff base ligand exerted low toxicity applied at concentrations up to 20 µM during 48 h on MDA-MB-231 breast cancer cells; complex 1 showed low effect at 20 µM concentration against A549 cells, while complex 2 exhibited stronger cytotoxicity on A549 cells with IC50 value of 17.54 µM (ref. 58) showing strong cytotoxicity in contrast to complexes examined in our study. In addition, four Zn(n) complexes derived from bidentate hydrazones also demonstrated higher intensity of cytotoxic activity against tested A549 lung cancer cells in comparison with activity of complexes tested in our study (IC50 values from 9.23 µM to 77.57 µM for 24 h treatment).59

Investigation of cytotoxic effects against normal fibroblasts MRC-5 represent a strength of current study, since selectivity in the cytotoxic activity of novel compounds against cancer cells in comparison with normal healthy cells is essential for evaluation of novel bioactive and anticancer compounds.

**Effects of Zn(n) complexes on HeLa cell cycle phase distribution**

To further explore the mechanisms of cytotoxic activity of three newly synthesized Zn(n) complexes, their effects on cell cycle phase distribution after 24 h incubation with IC50 and 2IC50 concentrations were examined in HeLa cells. As can be observed from Fig. 8, each of the three complexes induced a concentration-dependent increase in the percentage of HeLa cells in the subG1 phase compared to this percentage in control cells. Complex 3 applied at IC50 concentration caused a higher increase in the percentage of dead HeLa cells than complexes 1 and 2 applied at IC50 concentrations (10.05% vs. 6.75% and 6.10%). On the other hand, complex 1 at 2IC50 concentration induced the highest increase in the percentage of HeLa cells within the subG1 phase in comparison with complexes 2 and 3 applied at 2IC50 concentrations (27.61% vs. 19.40% and 18.60%).

![Fig. 8 Changes in the cell cycle phase distribution of HeLa cells treated with Zn(n) complexes 1, 2, and 3 applied at IC50 (A) and 2IC50 concentrations (B) during 24 h. C – control. The data shown represent the average ± standard deviation of two independent experiments.](image)
Effects of Zn(II) complexes on HeLa cell death

The possible cell death mode activated by complexes was further investigated in HeLa cells. Each of the compounds applied at IC_{50} concentration caused a small increase in the percentage of early apoptotic and late apoptotic/secondary necrotic HeLa cells in comparison with the control cell sample (Fig. 9). In addition, complex 3 increased the percentage of dead HeLa cells, stained with PI only. Treatment of HeLa cells for 24 h with 2IC_{50} concentrations of three Zn(II) complexes induced an increase in the percentage of cells in early apoptosis (7.45%, 4.69%, and 2.39% for complexes 1, 2, and 3 vs. 0.28% in control cells), late apoptosis/secondary necrosis (3.36%, 4.24%, and 10.55% for complexes 1, 2, and 3 vs. 0.69% in control cells), and in the percentage of dead cells stained with PI only when compared with these percentages in control cells (16.05%, 9.57%, and 36.18% for complexes 1, 2, and 3 vs. 3.48% in control cells). These results indicate the ability of each of the three investigated Zn(II) complexes to trigger apoptosis in HeLa cells. However, they might also activate cell death through other mechanisms besides apoptosis.

Effects of Zn(II) complexes on gene and microRNA expression levels in HeLa cells

After the treatment of HeLa cells with complex 1, the level of MMP9 expression was 1.92 times higher than in control cells, the level of miR-10b decreased by about 18%, the level of miR-34a increased by 22% compared with the control (Fig. 10). Treatment of HeLa cells with complex 1 nearly doubled the level of MMP9 resulting in a 92% increase, while levels of VEGFA decreased by about 31%. Treatment with complex 2 upregulated miR-10b by 79%, miR-34a by 11%, MMP9 by 87%, but downregulated VEGFA levels by 30%. Treatment with complex 3 increased suppressive miR-10b and miR-34a by 3.31 times, 55% respectively, then negligible increased MMP9 by 6%, and prominently decreased levels of VEGFA by 47%.

Metalloproteinase 9 and VEGFA were associated with tumor formation and progression in a wide variety of malignancies, including cervical carcinoma, while miR-34a predominantly acts as a tumor suppressive silencer of various oncopgenes.\textsuperscript{60,61} Furthermore, it has been shown that miR-34a negatively regulates MMP9 levels in glioma cells, so it may be logical to expect that if miR-34a-MMP9 axis is targeted by the complex, in the cases of miR-34a rise, MMP9 levels should fall. In our experiment, that was not the case, but if we compare the potential influence of complexes 1 and 2 on the one hand and complex 3 on the other, we can observe that miR-34a levels were higher, and MMP9 levels were lower after complex 3 treatment than after the treatment with complexes 1 or 2. MicroRNA miR-10b was described as a tumor suppressor and oncogene in various malignant tumors, but in cervical carcinoma, it has been reported to have a tumor-suppressive role.\textsuperscript{62}

Treatment with complexes 1 and 2 increased MMP9 levels but prominently lowered VEGFA. Complex 2 increased miR-10b levels while complex 1 decreased miR-10b, indicating that it has a less favorable signature among the investigated molecules. Complex 3 lowered substantially VEGFA, marginally increased MMP9, and highly increased miR-10b, and miR-34a levels. The structure of complex 3 may also be considered to be modified to maintain its effect and, at the same time to acquire better cytotoxic activity, as well as for complex 2 to change its biological activity in terms of MMP9 regulation.

Effects of Zn(II) complexes on intracellular ROS levels in MRC-5 cells

Each of the three tested Zn(II) complexes decreased the levels of ROS in MRC-5 cells incubated for 24 h with subtoxic IC_{20} concentrations of complexes 1, 2, and 3 in comparison with ROS levels in control, untreated MRC-5 cells (100 µM for complexes 1 and 2, 150 µM for complex 3) (Fig. 11). Complex 1 and complex 3 decreased the ROS levels for 35.49% and 42.49% in comparison with control MRC-5 cells, while complex 2 was less effective causing decrease for 18.95%. Since three Zn(II)
complexes exerted antioxidant effects, their cytoprotective potential against the production of ROS caused by short incubation with hydrogen peroxide was investigated. In contrast with previous results, complex 1 did not show an ability to reduce levels of oxidative stress in MRC-5 cells generated using hydrogen peroxide, while the level of oxidative stress was higher in cells incubated with complex 3 before exposure to hydrogen peroxide.

However, complex 2 remarkably reduced ROS levels in MRC-5 cells exposed to hydrogen peroxide compared to control cells exposed to hydrogen peroxide, confirming the antioxidant and cytoprotective potential of this Zn(n) complex.

Antimicrobial activity
The antimicrobial activity of the synthesized complexes, corresponding ligand and salts was evaluated against a panel of four Gram-positive and four Gram-negative bacterial strains, two fungi and one yeast strain. The MIC values are presented in Tables 6 and 7. The synthesized complexes show weak antibacterial activity compared to ampicillin. However, a very potent and selective antifungal activity is observed against all the strains tested. The antifungal activity of all tested complexes is comparable to the control antifungal agent Amphotericin B; for example, the MIC value of complex 3 against A. brasiliensis is only two times lower than the MIC value of the control compound. In view of the increasing demand for selective antifungal drugs, the mechanism of action of the synthesized complexes is being further investigated by Molecular docking studies.

Molecular docking
Since many known antymycotic drugs act as inhibitors of enzymes essential for biosynthesis of sterols63 and the complexes have significant antifungal activity against yeast strain S. cerevisiae, the six S. cerevisiae enzymes included in the biosynthetic sterol pathway were selected for molecular docking analysis with synthesized Zn complexes. The selected enzymes are: HMG-CoA reductase (UniProtKB P12683), Geranylgeranyl pyrophosphate synthase-GPPS (Q12051), Squalene synthase – SQS (P29704), Squalene epoxidase – SQE (P32476), sterol 24-C-methyltransferase-SMT (P25087) and Lanosterol 14α-demethylase-CYP51 (P10614). The predicted binding energy values at enzyme active sites are given in Table 8.

As shown in Table 8, none of the complexes can bind in the active site of HMG-CoA reductase, SQE, and SMT enzymes, so only non-specific binding sites on the surface of the proteins have been found. The smaller penta-coordinated complexes 2 and 3 bind in the active site of SQS and CYP51, while the

Table 6 Antibacterial activity of Zn(n) complexes and their precursor compounds

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<th>3</th>
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<th>NaOCN</th>
<th>Zn(BF4)2·6H2O</th>
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<td>3.60</td>
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DMSO served as a negative control.

Table 7 Antifungal activity of Zn(n) complexes and their precursor compounds

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<td>2.40</td>
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</table>
larger six-coordinated complex 1 is too large to fit in the active site of these enzymes. Our docking analysis shows that only the active site of GPPS is large enough to accommodate all three studied complexes. Furthermore, the binding energies for complexes 2 and 3 within the GPPS active site are significantly higher than for other enzymes. The best binding poses for the studied complexes in the GPPS active site and the 2D interaction diagram for the binding of these complexes are shown in Fig. 12.

It is worth noting that the GPPS enzyme has Mg$^{2+}$ in the active site, with which both the ambidentate NCO$^-$ (complex 2) and N$_3^-$ (complex 3) ligands form a coordinative bond. These interactions are absent in complex 1 (Fig. 12 right side), resulting in a lower overall binding energy (Table 8) and explaining the difference in measured antifungal activity. Other important interactions between the complexes and the active site amino acids include: (i) attractive electrostatic interactions between Asp and Glu residues on one side and Zn$^{2+}$ and quaternary ammonium from the ligand on the other; (ii) π–cation and π–anion interactions and (iii) halogen interactions between Asp residue and bromide.

Considering that MIC values for S. cerevisiae are comparable for all three tested complexes, molecular docking results predict that Zn(II) complexes exhibit antifungal activity, most likely via inhibition of the GPPS enzyme. Analysis of molecular docking results on other unique enzymes are presented in ESI (Fig. S18–S21$^\dagger$).

### Conclusions

Complexes with ligands based on 2-acetylpyridine and its derivatives have attracted attention in medicinal chemistry due to their diverse biological activities, including anticancer, antibacterial and antifungal activities. Therefore, a ligand with Girard’s T reagent and a 2-acetylpyridine derivative were selected for the subsequent synthesis of complexes with zinc ions and the evaluation of their biological activity.

X-ray crystallographic analysis, elemental analysis, IR and NMR spectroscopy, and DFT calculations were used to interpret and analyze obtained complexes 1–3. The hydrazone ligand in all three complexes is coordinated in a deprotonated form by the imine nitrogen, pyridine nitrogen, and carbonyl oxygen atoms. The six-coordination geometry of complex 1 is described as a distorted octahedral geometry, while complexes 2 and 3 exhibit five-fold coordination with a distorted trigonal bipyramidal geometry. Experimental results have shown that a simple change in the molecular ratio of the azide leads to the formation of complex 1 (when the use of N$_3^-$ is 4 times higher) or complex 3 (when the use of N$_3^-$ is 12 times higher). Calculated thermodynamic data indicate that all three complexes are stable in solution and retain the same coordination geometry as in the crystal structures. Furthermore, since OCN$^-$
is an ambidentate ligand, the preferred coordination via nitrogen is confirmed by thermodynamic stability calculations of the Gibbs free energy. The EDA-NOVC analysis explains this result by a stronger $\sigma$-covalency for coordination via nitrogen. Further insight into the electronic structure of the studied species is obtained from conceptual DFT, confirming the enhanced (re)activity of the complexes compared to the free ligand.

Cytotoxic activity was determined against five cancer cell lines and one normal cell line. The results showed that human chronic myelogenous leukemia K562 cells were the most sensitive to the cytotoxic effects of three new Zn(II) hydrazone complexes 1–3. Cervical adenocarcinoma HeLa cells and colorectal adenocarcinoma LS 174T cells were similarly sensitive to the cytotoxic effects of complexes 1 and 2, while breast adenocarcinoma MDA-MB-231 cells were most sensitive to the cytotoxicity of complex 2. The mechanisms of anticancer effects of the complexes were further investigated in cervical adenocarcinoma HeLa cells, while their potential antioxidant effects were investigated in normal fibroblasts. The investigated complexes induced cell death in HeLa cells and decreased vascular endothelial growth factor A (VEGFA) gene expression levels, which are involved in angiogenesis and further cancer progression. Complex 2 exerted prominent antioxidant and cytoprotective effects, as confirmed by remarkably reduced ROS levels in MRC-5 cells exposed to hydrogen peroxide compared to control cells. Although the synthesized compounds have no significant anticancer activity, these studies have increased our knowledge of the anticancer mechanism of Zn(II) complexes, which may lead to the rational design of highly selective and effective therapeutics with fewer side effects.

The antimicrobial activity of the investigated complexes was tested against two fungal strains, one yeast strain, four Gram-positive bacteria, and four Gram-negative bacteria. While the complexes did not show antibacterial activity, they did show a pronounced preference for fungal strains. All three complexes showed significant antifungal activity against A. brasiliensis activity against the yeast S. cerevisiae. A molecular docking study performed on six fungal enzymes important for sterol biosynthesis predicted that Zn(II) complexes exhibit antifungal activity, presumably via inhibition of S. cerevisiae GPPS. The results obtained suggest that zinc complexes act as selective antifungal drugs and further investigation is needed to fully understand their potential application. This is of great importance in view of the increasing demand for effective antymycotics.

**Experimental**

**Materials and methods**

All chemicals and solvents (reagent grade) have been purchased from commercial suppliers (NaN$_3$ from Riedel-de-Haën; all other chemicals from Sigma-Aldrich) and utilized without additional purification. Using the Elementar Vario ELIII C.H.N.S.O analyzer, standard micro-methods were implemented to perform elemental analyses (C, H, and N). Using the ATR technique, IR spectra were obtained from 400–4000 cm$^{-1}$ (strong – s, medium – m, weak – w) by a Nicolet 6700 FT-IR spectrometer. An NMR 400 MHz – Varian/Agilent spectrometer was used to record NMR spectra in deuterated dimethyl sulfoxide (DMSO-d$_6$). An Agilent Cary 3500 UV-Vis spectrophotometer was used to record the UV-Vis spectra.

**Synthesis**

**Synthesis of ligand (HCl).** (E)-2-[(1-(6-Bromopyridin-2-yl)ethylidene)hydrazinyl]-N,N,N-trimethyl-2-oxyothan-1-aminium chloride. In a reaction between 0.60 g of 2-acetyl-6-bromopyridine and 0.50 g of Girard’s T reagent (3.0 mmol) in 15 mL of methanol, the ligand HLCl was formed. The reaction mixture was acidified with two drops of glacial acetic acid (CH$_3$COOH) and refluxed for two hours at 70 ℃. Since the starting component 2-acetyl-6-bromopyridine is light-sensitive, the reaction was performed in a darkened balloon. After evaporation of the reaction solution in a vacuum evaporator, the ligand was obtained as a white solid. Yield: 0.67 g (64%). Elemental anal. calc. for C$_{12}$H$_{18}$BrClN$_3$: N, 16.02; C, 41.22; H, 5.19. Found: N, 16.55; C, 41.36; H, 5.13%.

IR (cm$^{-1}$): 3377.4 (m), 3068.5 (m), 2976.5 (w), 2940.6 (w), 2879.9 (w), 1703.4 (vs), 1618.3 (w), 1571.8 (m), 1550.4 (m), 1491.2 (m), 1481.9 (w), 1457.5 (m), 1398.4 (s), 1333.1 (m), 1280.2 (s), 1231.0 (m), 1164.0 (m), 1125.8 (s), 1094.3 (w), 1079.1 (w), 987.3 (m), 947.3 (w), 818.5 (m), 800.1 (m), 655.4 (m). 1H NMR: 11.46 (N–H, s). 8.11 (C3–H, d), 7.82 (C4–H, t), 7.68 (C5–H, d), 4.88 (C10–H$_2$, s), 3.32 (C11–H$_3$, s), 2.47 (C8–H$_3$, s). 13C NMR: 12.69 (C8), 53.69 (C11), 63.15 (C10), 120.17 (C3), 128.96 (C5), 140.38 (C4), 140.74 (C6), 149.52 (C7), 156.23 (C2), 167.18 (C9).

**Synthesis of [ZnL](BF$_4$)$_2$ complex 1.** Into the solution of ligand HCl (0.087 g, 0.25 mmol) in methanol (20 mL), Zn (BF$_4$)$_2$·6H$_2$O (0.087 g, 0.25 mmol) dissolved in water (10 mL) and solid NaN$_3$ in excess (0.065 g, 1 mmol) were added. The reaction mixture was heated to 70 ℃ for two hours with stirring. Yellow crystals appropriate for X-ray examination were obtained following a gradual solvent evaporation process at 4 ℃ for nearly three months. Elemental anal. calc. for C$_{22}$H$_{17}$Br$_3$BF$_5$N$_2$O$_3$: Zn: N, 14.12; C, 37.84; H, 4.70. Found: N, 14.20; C, 37.49; H, 4.61. IR (cm$^{-1}$): 1626.0 (w), 1583.0 (s), 1528.8 (s), 1491.7 (w), 1413.0 (m), 1390.2 (m), 1373.0 (s), 1344.1 (m), 1309.5 (m), 1252.9 (w), 1029.3 (s), 918.2 (m), 798.5 (m), 733.6 (m). 1H NMR: 2.61 (C8–H$_3$, s), 3.14 (C11–H$_4$, s), 4.08 (C10–H$_3$, s), 7.81 (C5–H, d), 8.08 (C4–H, t), 8.12 (C3–H, d). 13C NMR: 14.92 (C8), 54.54 (C11), 67.98 (C10), 124.46 (C9), 132.10 (C10), 120.17 (C3), 128.96 (C5), 140.38 (C4), 140.74 (C6), 149.52 (C7), 156.23 (C2), 167.18 (C9).

**Synthesis of [ZnL(NCO)$_2$] complex 2.** Into the solution of ligand HCl (0.087 g, 0.25 mmol) in methanol (20 mL), Zn (BF$_4$)$_2$·6H$_2$O (0.087 g, 0.25 mmol) dissolved in water (10 mL) and solid NaOCl in excess (0.065 g, 1 mmol) were added. The reaction mixture was heated to 70 ℃ for two hours with stirring. Yellow crystals suitable for X-ray examination were...
acquired following a gradual solvent evaporation process at 4 °C for nearly three months. Elemental anal. calc. for C_{13}H_{20}BrN_{10}O_{2}Zn: N, 17.59; C, 37.72; H, 4.22. Found: N, 17.81; C, 37.51; H, 4.28. IR (cm⁻¹): 3537.5 (w), 3104.5 (w), 2965.4 (w), 2209.3 (s), 1652.0 (w), 1559.2 (s), 1530.2 (s), 1492.0 (m), 1390.2 (m), 1373.3 (s), 1344.5 (m), 1309.8 (m), 1189.1 (m), 1061.2 (s), 1027.8 (s), 919.1 (m), 824.6 (m), 799.8 (m). ¹H NMR: 2.51 (C8–H, 3H, s), 3.24 (C11–H, 9H, s), 4.11 (C10–H, 2H, s), 7.80 (C3–H, 1H, dd), 7.95 (C4–H, 1H, t), 8.10 (C3–H, 1H, dd). ¹³C NMR: 13.91 (C8), 53.76 (C11), 66.83 (C10), 122.79 (C3), 125.62 (C12), 131.03 (C5), 141.92 (C4), 142.32 (C6), 151.40 (C2), 152.03 (C7), 170.52 (C9).

**Synthesis of [Zn(NL)_2] complex** 3. Into the solution of ligand HCl (0.087 g, 0.25 mmol) in methanol (20 mL), solid Zn(BF₄)₂·6H₂O (0.087 g, 0.25 mmol) dissolved in water (10 mL) and solid NaN₃ in excess (0.195 g, 3 mmol) were added. At 70 °C for five hours, the reaction mixture was stirred while heating. After nearly two months-long slow evaporation of the solvent in a refrigerator at 4 °C, white crystals appropriate for X-ray examination were obtained. Elemental anal. calc. for C_{13}H_{20}BrN_{10}OZn: N, 29.32; C, 32.69; H, 4.22. Found: N, 29.43; C, 32.85; H, 4.27. IR (cm⁻¹): 3370.3 (w), 3092.2 (w), 3037.4 (w), 2060.4 (vs), 1612.0 (w), 1562.6 (m), 1536.1 (m), 1486.1 (w), 1431.5 (w), 1408.6 (m), 1341.8 (m), 1310.1 (w), 1313 (m), 1290.1 (w), 1120.6 (w), 1070.1 (w), 1020.6 (w), 824.6 (m), 794.6 (w). ¹H NMR: 2.51 (C8–H, 3H, s), 3.23 (C11–H, 9H, s), 4.12 (C10–H, 2H, s), 7.85 (C5–H, 1H, d), 7.97 (C4–H, 1H, t), 8.01 (C3–H, 1H, d). ¹³C NMR: 13.69 (C8), 53.74 (C11), 66.84 (C10), 122.78 (C3), 130.89 (C5), 141.78 (C4), 142.60 (C6), 151.78 (C2), 151.80 (C7), 170.86 (C9).

**X-Ray structure determination**

The crystal-structures of the complexes [ZnL₂][BF₄]₂, [ZnL(NCO)₂], and [ZnL(N₃)₂] were determined by single-crystal X-ray diffraction method. Crystallographic data and refinement details are given in Table 9. Diffraction data were collected with Agilent SuperNova dual source diffractometer using an Atlas detector and equipped with mirror-monochromated Mo-Kα radiation (λ = 0.71073 Å) at room temperature for [ZnL(N₃)₂] and at 150 K for [ZnL(NCO)₂] and [ZnL₂][BF₄]₂, respectively. The data were processed by using Crysalis PRO.⁶⁴ The structures were solved with Olex software⁶⁵ using SHELXT⁶⁶ and refined by full-matrix least-squares base on F² using SHELXL.⁶⁷ All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were included in the model at geometrically calculated positions and refined using a riding model. The C14 and O3 atoms of one NCO ligand in [ZnL(NCO)₂] are disordered over two orientations and were refined with the use of the PART instructions. The occupancy of C14a and C14b or O3a and O3b refined to the ratio of 58 and 42%, respectively.

CCDC 2339925–2339927 contains the supplementary crystallographic data for this paper.†

**Antimicrobial activity**

*In vitro*, antibacterial and antifungal activity was tested against four Gram-positive bacteria (*Bacillus spizizenii* ATCC 6633, *Clostridium sporogenes* ATCC 19404, *Micrococcus luteus* ATCC 10240, *Staphylococcus aureus* ATCC 6538), four Gram-negative bacteria (*Proteus hauseri* ATCC 13315, *Escherichia coli* ATCC

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**Table 9** Crystal data and structure refinement details for [ZnL₂][BF₄]₂, [ZnL(NCO)₂] and [ZnL(N₃)₂]

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<td>+1.16/−1.08</td>
<td>+2.30/−0.79</td>
<td>+0.58/−0.60</td>
</tr>
</tbody>
</table>

* R = Σ||F_o|−|F_c||/Σ|F_o|, δ_wR₂ = Σ[w(F_c² − F_o²)]/Σ[w(F_o²)]¹/², S = Σ[|F_o² − F_c²|²]/[n(p)], where n is the number of reflections and p is the total number of parameters refined.

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25922, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumoniae* ATCC 10031), and two fungal strains (*Aspergillus brasiliensis* ATCC 16404, *Candida albicans* ATCC 10231) and one yeast strain (*Saccharomyces cerevisiae* ATCC 9763), by the double dilution method in microtiter plates. Antibacterial activity was determined using Mueller–Hinton broth, whereas antifungal activity was determined using Sabouraud dextrose broth. The concentration of stock solutions of the tested compounds was 10 mg mL\(^{-1}\) in DMSO. After adding 100 μL of the appropriate broth to the microtiter plate, 100 μL of the compounds were added and double diluted. A suspension of bacteria and fungi was prepared in sterile 0.9% saline, and 10 μL of diluted suspension was added to each well to obtain a final concentration of 5 × 10\(^5\) CFU mL\(^{-1}\) for bacteria and 5 × 10\(^3\) CFU mL\(^{-1}\) for fungi. Ampicillin served as a positive control for bacteria, amphotericin B served as a positive control for fungi, whereas DMSO served as a negative control. Microtiter plates were incubated for 24 h at 37 °C for bacteria and for 48 h at 28 °C for fungi. After incubation, 10 μL of 10% solution of resazurin was added for visualization of bacterial growth. The minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibited bacterial growth (the blue color of resazurin exists) and is given in mM concentration.

**DFT computational details**

All DFT calculations were done with the ADP\(^{68}\) engine in the Amsterdam Modeling Suite (version 2023.104).\(^{59}\) The all-electron triple-zeta Slater-type orbitals plus one polarization function (TZP) basis set was used for all atoms. The scalar-relativistic Zeroth Order Regular Approximation (ZORA) was used in all calculations to account for relativistic effects.\(^{70-72}\) Geometry optimizations were performed with the BP86 exchange–correlation functional,\(^{42-44}\) with Grimme’s fourth generation dispersion energy corrections,\(^{45}\) i.e., BP86-D4. The COSMO solvation model,\(^{73,74}\) as implemented in ADP,\(^{46}\) was used to consider solvent effects. Water, methanol, and DMSO solvents were considered in geometry optimizations. The harmonic frequencies were calculated at the same level of theory. Low vibrational frequency free rotor interpolation correction proposed by Li/Head-Gordon\(^{75}\) and Grimme\(^{76}\) has been used to evaluate internal energies and entropic effects to the Gibbs free energy at 298 K (frequency cut-off 100 cm\(^{-1}\)). Correction due to the standard state conversion (1 atm. to 1 mol dm\(^{-3}\) solution standard state) is applied, leading to the correction of 1.89 kcal mol\(^{-1}\) to the free energies at 298 K. In reactions involving a molecule that is a solvent (water, methanol, DMSO), the free energy correction due to the conversion to the solvent standard state is made (4.27, 4.13, 3.46 kcal mol\(^{-1}\) at 298 K, for water, methanol, and DMSO respectively). Electronic energies used to calculate the Gibbs free energy were evaluated with range-separated hybrid CAM-B3LYP\(^{47}\) functional at ZORA-BP86-D4/TZP-COSMO geometries.

The electronic Chemical Potential (\(\mu\)), the molecular hardness (\(\eta\)), the molecular softness (\(S\)), and the electrophilicity index (\(\omega\)) (Conceptual DFT global descriptors) were calculated in the finite difference linearization (FDL) approximation,\(^{50}\) based on the calculations of the electronic energy of a molecule studied and the molecule with one more and one less electron (\(N, N+1, \text{and } N-1\) electron systems):

\[
\mu = \frac{E_e(N + 1) - E_e(N - 1)}{2} \tag{1}
\]

\[
\eta = E_e(N + 1) - 2E_e(N) + E_e(N - 1) \tag{2}
\]

\[
S = \frac{1}{\eta} \tag{3}
\]

\[
\omega = \frac{\mu^2}{2\eta} \tag{4}
\]

CAM-B3LYP\(^{47}\) functional, TZP basis set, and COSMO solvation model with water as solvent were used for Conceptual DFT calculations at ZORA-BP86-D4/TZP-COSMO(water) geometries. Unrestricted formalism was used for calculations of energies of complexes with one electron more or less, as needed for the FDL approach.

The LibXC library\(^{77}\) was used for all calculations employing CAM-B3LYP functional.

Chemical bonding between fragments \([\text{ZnL}]^{2+}_{\eta}\text{L}_n[\text{ZnL}]^{2+}_{\eta}\text{L}_n(\text{NCO})_2^{2-}\text{L}_n[\text{ZnL}]^{2+}_{\eta}\text{L}_n(\text{OCN})_2^{2-}\) was analyzed by the extended transition state Energy Decomposition Analysis (EDA)\(^{51-53}\) as implemented in ADF program package, at ZORA-BP86-D4/TZP level of theory on corresponding geometries. The interaction energy between fragments is decomposed into \(E_{\text{int}} = E_{\text{elec}} + E_{\text{Pauli}} + E_{\text{orb}} + E_{\text{disp}}\). Additionally, natural orbitals for chemical valence (NOCV)\(^{54,55}\) decomposition of the electron density deformation was performed to elucidate different density transfer channels and to quantify their importance as an energy contribution to the \(E_{\text{orb}}\). The charge flow between the fragments was quantified using Hirshfeld charge analysis.\(^{78}\)

To facilitate data exchange and dissemination, according to the FAIR principles\(^{79}\) of Open Data sharing, a data set collection of computational results is available in the ioChem-BD repository\(^{80}\) and can be accessed via [https://doi.org/10.19061/iochem-bd-6-362](https://doi.org/10.19061/iochem-bd-6-362).

**Determination of cytotoxic activity by MTT assay**

Cytotoxic activities of three novel Zn(II) complexes and their precursor compounds were examined against five human cancer cell lines: cervical adenocarcinoma HeLa, lung carcinoma A549, breast adenocarcinoma MDA-MB-231, chronic myelogenous leukemia K562, colorectal adenocarcinoma LS 174T, and against normal human lung fibroblasts MRC-5. The human cell lines were purchased from the American Type Culture Collection (Manassas, VA, USA). The cells were seeded into 96-well cell culture plates in RPMI-1640 medium at a seeding density of 2000 cells per well for HeLa cells, 5000 cells per well for A549, MDA-MB-231, K562, and MRC-5 cells, and 7000 cells per well for LS 174T cells. Cell lines were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 25 mM HEPES, and penicillin-streptomycin solution. Plates with cells were incubated at 37 °C with 5% CO\(_2\) and humidified air, as described else-
where. The cells were treated with five different concentrations of tested complexes and precursor compounds (12.5, 25, 50, 100, and 200 μM) during 72 h. Following 72 h incubation, the cell survival was measured by MTT colorimetric assay using the protocol established by Mosmann, modified by Ohno and Abe, and described previously. The absorbance was measured at 570 nm using the Thermo Scientific Multiskan SkyHigh microplate spectrophotometer. Three independent experiments were performed in triplicate.

All reagents for cell culture experiments were purchased from Sigma Aldrich, while 96-well cell culture plates were purchased from Thermo Scientific.

**Cell cycle analysis by flow cytometry**

HeLa cells (200 000 cells per well) were seeded in RPMI-1640 complete nutrient medium in 6-well cell culture plates and treated with IC50 and 2IC50 concentrations of three Zn(II) complexes for 24 h. After 24 h exposure, treated and control cells were collected by trypsinization, washed with phosphate-buffered saline (PBS) and fixed in cold 70% ethanol on ice, according to the protocol described by Ormerod. Before cell cycle analysis, the cells were collected by centrifugation, resuspended in PBS with RNase A and incubated for 30 min at 37 °C. Following incubation, the propidium iodide (PI) solution was added to cells. Cell cycle analysis was performed using a BD FACSCalibur™ flow cytometer (BD Biosciences) and BD CellQuest™ Pro software. Two independent experiments were performed.

Chemicals used for cell cycle analysis were products of Sigma Aldrich, while Thermo Scientific™ Biolite™ 6-well plates for adherent cells were used.

**FITC Annexin V/propidium iodide staining flow cytometric assay**

HeLa cells (200 000 cells per well) were seeded in RPMI-1640 complete nutrient medium into 6-well cell culture plates and treated with IC50 and 2IC50 concentrations of three Zn(II) complexes for 24 h. After 24 h incubation, HeLa cells were collected by trypsinization, washed with PBS, and stained with FITC Annexin V and propidium iodide for 15 minutes at room temperature in the dark, according to the assay procedure recommended by the manufacturer. The percentages of Annexin V− PI− viable cells, Annexin V− PI+ early apoptotic cells, Annexin V+ PI− late apoptotic/secondary necrotic cells, and Annexin V+ PI+ dead cells were detected using BD FACSCalibur™ flow cytometer and BD CellQuest™ Pro software for acquisition and analysis. Two independent experiments were performed.

FITC Annexin V and Annexin V Binding Buffer were products of BD Pharmingen, the other used cell culture reagents, including propidium iodide were products of Sigma Aldrich; Thermo Scientific™ Biolite™ 6-well plates were used.

**Gene and microRNA expression analyses by RT-qPCR**

The total RNA containing both, mRNAs and microRNAs was isolated from HeLa cells treated with IC50 concentrations of Zn (n) complexes 1, 2, and 3 and from control cells by following the manufacturer’s instruction from TRI Reagent (Sigma Aldrich) protocol. One ml of TRI Reagent was used to disrupt cells and was followed by the addition of 0.2 ml of chloroform, precipitated with 0.5 ml of isopropanol, and washed with 75% Et–OH diluted in Ambion nuclease-free water. The RNA concentrations were measured by BiospecNano (Shimadzu Corporation, Japan). High-Capacity cDNA Reverse Transcription Kit in the cases of cDNA for gene expression evaluation was used for transcription of 1 μg of RNA into cDNA, while MicroRNA Reverse Transcription Kit Applied Biosystems by Thermo Fisher Scientific, Lithuania was used for transcription of 10 ng miR-10b and miR-34a, and small nucleolar RNU6B as endogenous control (ID002218, ID000426, and ID001093, respectively). Quantitative real-time PCR (RT-qPCR) was performed with 2xTaqMan Universal Master Mix No UNG to amplify miR-10b/34a, MMP9 (Hs00957562_m1), and VEGFA (Hs00900055_m1) molecules. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with assay ID Hs02758991_1_g1 was used as an endogenous control for MMP9 and VEGFA normalization, and the comparative delta-delta threshold cycle (ddCt) method was used to obtain relative quantity values (RQ). The treated samples were calibrated to the non-treated control. The reaction of RT-qPCR was performed on QuantStudio™ 5 Real-Time PCR System (Applied Biosystems), while QuantStudio™ Design and Analysis Software (v.1.5.1) was used to calculate RQ values.

**Measurement of intracellular levels of reactive oxygen species by flow cytometry**

MRC-5 cells (200 000 cells per well) were seeded in RPMI-1640 complete nutrient medium in 6-well cell culture plates and treated for 24 h with subtoxic IC20 concentrations of complexes 1, 2, and 3 (100 μM for complexes 1 and 2, 150 μM for complex 3). Following 24 h incubation, MRC-5 cells were collected by trypsinization, washed with PBS, and stained with 30 μM cell permeable 2',7'-dichlorodihydrofluorescein diacetate for 45 min at 37 °C, as described earlier. The fluorescence intensity of dichlorofluorescein in MRC-5 cells as a measure of intracellular reactive oxygen species (ROS) levels was determined using BD FACSCalibur™ flow cytometer and BD CellQuest™ Pro software for acquisition and analysis. For examination of cytoprotective properties of Zn(II) complexes, control MRC-5 cells and cells incubated for 24 h with complexes were exposed for 20 min to 5 mM hydrogen peroxide solution (H2O2) in PBS at 37 °C, before determination of ROS levels. Two independent experiments were performed.

Chemicals used for ROS analysis were products of Sigma Aldrich, while Thermo Scientific™ Biolite™ 6-well plates for adherent cells were used.

**Molecular docking**

**Ligands preparation.** Starting from ZORA-BP86-D4/TZP-COSMO(water) optimized geometries of complexes 1, 2 and 3, Mertz-Kollman charges were calculated with RESP87 procedure, using B3LYP86,89 hybrid functional and 6-31(d,p)90
basis set for non-metal atoms and lanl2dz\(^{91}\) basis set with effective core potential for Zn atom. In these calculations, the radius of Zn\(^{2+}\) ion was set to 1.0 Å. These calculations were done with Gaussian09\(^{92}\) and RESP 2.4\(^{93}\) programs. The pdbqt files of complexes suitable for docking experiment were initially created in AutoDockTools (version 1.5.6) program\(^{94}\) and previously calculated Mertz-Kollman charges were added manually.

Proteins preparation. Out of six investigated enzymes of \textit{S. cerevisiae} crystal structures of only two (GPPS and CYP51) were found in Protein Data Bank (PDB).

For GPPS, a crystal structure with PDBID 2dh4\(^{95}\) was extracted from PDB and water molecules were deleted from the structure, leaving catalytically important Mg\(^{2+}\) ion. From the crystal structure of CYP51 (PDBID: 8dl4), all heteroatoms were deleted except the HEM group.

For HMG-CoA reductase, SQS, SQE, and SMT enzymes, homology models created with two different modeling strategies (Swiss model repository\(^{96}\) and AlfaFold2\(^{97}\)) were used. Next, for all protein structures, the protonation state of each titratable amino acid was determined with H++ program,\(^{98}\) based on the finite difference Poisson–Boltzmann (FPDB) continuum electrostatics method. Finally, individual atomic Kollman charges and standard residue atom types needed for the docking calculations were added to the protein structures with the AutoDockTools\(^{94}\) program. For GPPS protein, the charge of Mg\(^{2+}\) ion was set to +2, and for the HEM group in CYP51, individual atomic charges were calculated with the Mertz-Kollman/RESP procedure described above and manually inserted in the pdbqt file.

The docking experiment was done with the Auto Dock 4.2\(^{99}\) program using the Lamarckian genetic algorithm with 150 individuals in population and 25,000,000 energy evaluations. Discovery Studio software\(^{100}\) was used to analyze and visualize the results of the docking study.

Author contributions

Data availability
The data supporting this article have been included as part of the ESI.\(^{†}\)

Crystallographic data for 1–3 has been deposited at the CCDC under 2339925–2339927.\(^{†}\)

Data for this article are available at the ioChem-BD repository and can be accessed via https://doi.org/10.19061/iochem-bd-6-362.

Conflicts of interest
There are no conflicts to declare.

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References

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