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Cu(II), Mn(II) and Zn(II) complexes of hydrazones with quaternary ammonium moiety: synthesis, experimental and theoretical characterization and cytotoxic activity

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Abstract

In this paper Cu(II), Mn(II) and Zn(II) complexes with N,N,N-trimethyl-2-oxo-2-(2-(1-(thiazol-2-yl)ethylidene)hydrazinyl)ethan-1-aminium chloride (HL1Cl) were synthesized and characterized by single-crystal X-ray diffraction, IR spectroscopy, elemental analysis and DFT calculations. In all three complexes ligand (L1) is coordinated in deprotonated formally neutral zwitter-ionic form via NNO donor set atoms. Cu(II) and Zn (II) form mononuclear penta-coordinated complexes [CuL1(N3)(CH3OH)]BF4 and [ZnL1(N3)2], respectively, while with Mn(II) a binuclear [Mn2L12(μ-1,1-N3)2(N3)2]·2CH3OH complex, with unusual distorted trigonal-prismatic geometry around metal centers, has been obtained. Antimicrobial activity was tested against a panel of Gram-negative and Gram-positive bacteria, two yeasts and one fungal strain. The binuclear Mn(II) complex showed antifungal activity of similar intensity as amphotericin B. Based on the results of the brine shrimp test and DPPH radical scavenging activity, the most active, Cu(II) and Mn(II) complexes, were selected for evaluation of cytotoxic activity against five malignant cancer cell lines (HeLa, A375, MCF7, PC-3 and A549) and one normal cell line HaCaT. Both complexes showed a significant activity. It should be pointed out that the activity of Mn(II) complex against breast cancer MCF7 cell is only slightly weaker than that of cisplatin, but with selectivity to the tumor cell line in comparison to normal HaCaT cells, which is non-existent in case of cisplatin.

Introduction

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Metal complexes with hydrazones have been investigated intensively during previous years due to their various pharmaceutical applications as antitumor, antibacterial, antiviral and antifungal agents. Of particular interest are hydrazone ligands with –CH=N-NH-C(O)– group, formed by condensation of aldehydes/ketones with different hydrazides. Girard’s T reagent (trimethylaminoacetohydrazide chloride) is attractive due to its ability to form water-soluble hydrazones with various aldehydes/ketones. Using aldehydes/ketones with thiazole ring in their structure for the synthesis of hydrazone ligands, additional coordination atoms are introduced. Combining both properties (additional coordination site due to thiazole ring and water-solubility due to positively charged quaternary ammonium moiety) in metal hydrazone complexes can lead to enhanced biological activity.

Copper, manganese and zinc are essential trace elements with many physiological functions. Ions of these elements act as cofactors and as allosteric components for many enzymes. Cu(II) and Mn(II) complexes have been studied as low molecular weight model compounds that mimic the active site of superoxide dismutase (SOD), which participate in cell oxidative stress regulation. According to the biological evaluations, complexes of Cu(II), Mn(II) and Zn(II) with Schiff base ligands can possess different biological activities, such as effective inhibition against bacteria and fungi, as well as cytotoxic activity.

Schiff base complexes of Cu(II) and Mn(II), both mononuclear and dinuclear, were previously reported. Most of biologically active copper and manganese complexes have mononuclear structure, with the most common square-planar and square-pyramidal geometry around Cu(II) ion and octahedral geometry around Mn(II) ion.

In continuation of our previous investigations on synthesis, characterization and biological activity of complexes with Girard’s T reagent-based hydrazones, which showed moderate biological activities, in this paper, a three novel Girard’s T reagent-based complex with Cu(II), Mn(II) and Zn(II) ions are described.

**Results**

**General**

The reaction of 2-acetylthiazole and Girard’s T reagent was performed according to the previously reported method and yielded the ligand N,N,N-trimethyl-2-oxo-2-(2-(1-(thiazol-2-yl)ethylidene)hydrazinyl)ethan-1-aminium chloride (HLCl), which was used for the synthesis of...
complexes 1–3 (Scheme 1). Reaction of the ligand $HL^1\text{Cl}$ with metal salts $\text{Cu(BF}_4\text{)}\times6\text{H}_2\text{O}$ / $\text{MnCl}_2\times4\text{H}_2\text{O}$ / $\text{Zn(BF}_4\text{)}\times6\text{H}_2\text{O}$ and $\text{NaN}_3$ in mole ratio $1 : 1 : 4$ in methanol results in formation of mononuclear Cu(II) complex (1) with composition $[\text{CuL}^1(\text{N}_3)(\text{CH}_3\text{OH})]\text{BF}_4$, binuclear Mn(II) complex 2, with composition $[\text{Mn}_{2}L^1\text{2(\mu}_{1,1}\text{-}\text{N}_3\text{2)}(\text{N}_3\text{2)}\text{2CH}_3\text{OH}$, and mononuclear Zn(II) complex (3) with composition $[\text{ZnL}^1(\text{N}_3\text{2})]$. The ligand is coordinated in a deprotonated formally neutral zwitter-ionic form via NNO donor set atoms in all three complexes.

\[\text{Cu(BF}_4\text{)}\times6\text{H}_2\text{O (1 equiv), NaN}_3\text{(4 equiv)} \rightarrow (1)\]
\[\text{MnCl}_2\times4\text{H}_2\text{O (1 equiv), NaN}_3\text{(4 equiv)} \rightarrow (2)\]
\[\text{Zn(BF}_4\text{)}\times6\text{H}_2\text{O (1 equiv), NaN}_3\text{(4 equiv)} \rightarrow (3)\]

\[\text{MnCl}_2\times4\text{H}_2\text{O (1 equiv), NaN}_3\text{(4 equiv)} \rightarrow (2)\]

\[\text{Zn(BF}_4\text{)}\times6\text{H}_2\text{O (1 equiv), NaN}_3\text{(4 equiv)} \rightarrow (3)\]

**Scheme 1** Synthesis of $[\text{CuL}^1(\text{N}_3)(\text{CH}_3\text{OH})]\text{BF}_4$ (1), $[\text{Mn}_{2}L^1\text{2(\mu}_{1,1}\text{-}\text{N}_3\text{2)}(\text{N}_3\text{2)}\text{2CH}_3\text{OH}$ (2) and $[\text{ZnL}^1(\text{N}_3\text{2})]$ (3) complexes.

**Spectroscopy**

**IR spectra.** The IR spectroscopy data confirm that the $HL^1\text{Cl}$ ligand (Fig. S4) is coordinated in a deprotonated form, since the $\nu$(N–H) band at 2955 cm$^{-1}$ is absent in the spectrum of all complexes. The presence of a medium, sharp peak at 3050 cm$^{-1}$ in the spectrum of 1 (Fig. S5), and a medium, broad peak at 3388 cm$^{-1}$ in the spectrum of 2 (Fig. S6), point to the coordination of methanol $\nu$(O–H) in the case of complex 1, and presence of methanol in the crystals of complex 2. In the IR spectra, a strong band at 2047 cm$^{-1}$, 2042 cm$^{-1}$ and 2057 cm$^{-1}$ originates from
coordinated N₃⁻ for complexes 1, 2 and 3 (Figs. S7-S8), respectively. In the case of complex 2 additional weak band at 2111 cm⁻¹ is assigned to bridging azido ligand. Instead of the ν(C=O) band at 1701 cm⁻¹, observed in the spectrum of the ligand HL¹Cl, the new bands at 1698 cm⁻¹, 1688 cm⁻¹ and 1690 cm⁻¹ appeared in the spectra of complexes 1, 2 and 3, respectively, being assigned to the ν(–O–C=N) vibrations of the deprotonated hydrazide moieties. Coordination of azomethine nitrogen atoms results in the shift of ν(C=N) band from 1612 cm⁻¹ in the spectrum of the ligand HL¹Cl to 1604 cm⁻¹, 1595 cm⁻¹, and 1600 cm⁻¹ in the spectra of complexes 1, 2 and 3, respectively.

NMR spectra. The signal of hydrazide NH at 11.86 ppm (Fig. S9) is absent in the ¹H NMR spectra of complex 3 (Fig. S10), indicating that the ligand is coordinated in deprotonated zwitter-ionic form. Coordination of thiazole nitrogen in the Zn(II) complex can be confirmed from a downfield shift of C3-H from 7.93 ppm in the spectrum of HL¹Cl to 8.04 ppm in the spectrum of Zn(II) complex. Due to the carbonyl oxygen atom's coordination, the carbonyl carbon (C6) signal is shifted downfield from 167.04 ppm in the spectrum of HL¹Cl (Fig. S11) to 171.59 ppm in the spectrum of complex 3 (Fig. S12). Downfield shift of azomethine carbon atom (C4) signal from 146.98 ppm in the spectrum of HL¹Cl to 147.32 ppm in the spectrum of Zn(II) complex indicates coordination of azomethine nitrogen. Coordination of thiazole nitrogen atom caused upfield shift of C3 atom signal from 143.94 ppm in the spectrum of HL¹Cl to 143.44 ppm in the spectra of Zn(II) complex.

Crystal structures of [CuL¹(N₃)(CH₃OH)]BF₄ (1), [Mn₂L¹₂(μ⁻1,1⁻N₃)₂(N₃)₂]·2CH₃OH (2) and [ZnL¹(N₃)₂] (3) complexes
The Cu(II), Mn(II) and Zn(II) ions with L¹ form mononuclear [CuL¹(N₃)(CH₃OH)]BF₄ (1) and [ZnL¹(N₃)₂] (3) and binuclear [Mn₂L¹₂(μ⁻1,1⁻N₃)₂(N₃)₂]·2CH₃OH (2) complexes in which L¹ in zwitter-ionic form coordinates as a tridentate ligand to M(II) ions through thiazole and imine nitrogen atoms and enolate oxygen atom. Complexes 1 and 3 crystallize in the monoclinic crystal system with space group No. 14 (P2₁/n and P2₁/c cell settings, respectively) and complex 2 in the triclinic crystal system with space group P–1 (No. 2).

Crystal structure of complex 1. The asymmetric unit (asu) of 1 consists of complex cation [CuL¹(N₃)(CH₃OH)]⁺ and BF₄⁻ anion. The molecular structure of the complex cation [CuL¹(N₃)(CH₃OH)]⁺ with atom numbering scheme is shown in Fig. 1. Selected bond distances
and valence angles are given in Table S1. The Cu(II) ion has fivefold coordination with in-plane coordinated $L^1$ through NNO-set of donor atoms and one nitrogen atom (N5) of the azide ligand, while the apical position is occupied by an oxygen atom (O2) from methanol. In general, the distortion in the five-coordinated systems is described by an index of trigonallity $\tau = (\beta - \alpha)/60$, where $\beta$ is the greatest basal angle and $\alpha$ is the second greatest angle. The parameter $\tau$ is 0 for regular square-based pyramidal forms and 1 for trigonal bipyramidal forms. The five-coordination geometry of the Cu(II) ion can be described as distorted square-based pyramidal, as indicated by the $\tau$ value of 0.26. The greatest basal angles N5–Cu1–N2 and O1–Cu1–N1 are 174.8(1)° and 159.05(9)°, respectively. The Cu(II) ion is lifted out by 0.1038(4) Å from the basal plane towards the apical ligand atom (O2). The dihedral angle of nearly 5.0° between two five-membered chelate rings (Cu–N–C–C–N and Cu–N–N–C–O) shows the non-coplanar nature of the metal-ligand system in $\mathbf{1}$. The Cu–N$_{\text{Ar}}$ 2.048(2) Å, Cu–N$_{\text{imine}}$ 1.928(2) Å and Cu–O$_{\text{enolate}}$ 1.970(2) Å bond lengths in $\mathbf{1}$ are comparable with those observed in the structurally related [Cu$L^2$Cl(BF$_4$)], [Cu$L^2$Cl(NO$_3$)] and [Cu$L^2$Cl(ClO$_4$)] complexes ($L^2$ = condensation product of 2-acetylpyridine and trimethylammoniumacetohydrazide chloride) of distorted square pyramidal geometry in which the apical ligands BF$_4^-$, NO$_3^-$, and ClO$_4^-$ are weakly coordinatively bound to Cu(II) ion. The axial Cu1–O2 2.533(2) Å bond in $\mathbf{1}$ is shorter than Cu–F(BF$_4^-$) 2.581(4) Å, Cu–O(NO$_3^-$) 2.607(2) Å and Cu–O(ClO$_4^-$) 2.73(1) Å axial bonds in [Cu$L^2$Cl(BF$_4$)] 35, [Cu$L^2$Cl(NO$_3$)] 35 and [Cu$L^2$Cl(ClO$_4$)] 34,35 complexes, respectively. In the crystals of $\mathbf{1}$, complex cations form hydrogen-bonded dimers at $\frac{1}{2}$ $\frac{1}{2}$ 0 and 0 0 $\frac{1}{2}$ through intermolecular O2–H2A···N5 hydrogen bond (Table S4 and Fig. S1). The counter anion (BF$_4^-$) mediates in joining the H-bonded dimers into the layers parallel with the (202) lattice plane through intermolecular C–H···F hydrogen bonds (Table S4 and Fig. S1a) and assists in connecting the neighboring layers by means of C–H···F hydrogen bonds (Table S4 and Fig. S1b). The Cu1···Cu1$^a$ (a = 1–x, 1–y, z) separation of 4.9983(7) Å observed in the H-bonded dimers is greater than those found in the pseudo-dimeric structures of the [Cu$L^2$Cl(BF$_4$)], [Cu$L^2$Cl(NO$_3$)] and [Cu$L^2$Cl(ClO$_4$)] complexes (3.5793–3.7973 Å) 34,35. However, in the crystal structure of $\mathbf{1}$, the shortest Cu···Cu$^b$ (b = –x, 1–y, –z) separation of 3.384 Å has been observed between the Cu(II) ions belonging to the neighboring (202) layers (Fig. S1b).

Crystal structure of complex 2. The crystal structure of $\mathbf{2}$ displays a centrosymmetric binuclear complex with the asymmetric unit comprising one Mn(II) center, one ligand $L^1$, two azide anions...
(one bridging and one terminal) and one solvent (methanol) molecule. The molecular structure of 
[Mn$_2$L$_2$(μ-1,1-N$_3$)$_2$(N$_3$)$_2$] with atom numbering scheme is shown in Fig. 2. Selected bond distances and valence angles are given in Table S2. The Mn(II) ion is hexacoordinated with three donor atoms N1, N2 and O1 of ligand L$, two nitrogen atoms (N5 and N5$^c$ were c is 1−x, 1−y, 1−z) from bridging azide anions, and one nitrogen atom (N8) from terminal azide anion. The polyhedron around the Mn(II) ion is described as a distorted trigonal prism (TPR-6) with the twist angle $\phi$ of 14.19° (mean value) being calculated applying the method 1 reported in Ref. 36 for the atom pairs N1N8, O1N2 and N5N5$^c$. Comparing with the octahedral Ni(II) complexes [Ni$_2$L$_2$(μ-1,1-N$_3$)$_2$(N$_3$)$_2$]·2H$_2$O and [Ni$_2$L$_2$(μ-1,1-N$_3$)$_2$(N$_3$)$_2$]·4H$_2$O 37 with the same ligand the [Mn$_2$L$_2$(μ-1,1-N$_3$)$_2$(N$_3$)$_2$]·2CH$_3$OH (2) complex shows significantly longer M–N$_Ar$ and M–N$_{imine}$ bond distances (Mn–N$_Ar$ 2.3668(19)Å, Ni–N$_Ar$ 2.122(2)–2.126(3) Å; Mn–N$_{imine}$ 2.2500(18)Å, Ni–N$_{imine}$ 1.997(2)–2.017(3)Å; Mn–O$_{enolate}$ 2.1879(16) Å and Ni–O$_{enolate}$ 2.083(2)–2.140(2) Å) indicating weaker coordination of L$^1$ to Mn(II) ion. Similarly, as in the corresponding Ni(II) complexes 37 in the complex 2, the terminal azido ligands are coordinated in trans positions. The N9–N8–Mn1 bond angle of 137.7(2)° shows bent coordination of the anionic terminals. The central Mn$_2$N$_2$ ring is planar with bridging angle Mn–N$_{azido}$ (end-on)–Mn$^c$ of 107.37(8)° and Mn···Mn$^c$ separation of 3.6031(6) Å. The Mn–N$_{azido}$ bond distances show a discrepancy of 0.0135 Å. In the crystals of 2, binuclear complex units are connected by weak intermolecular C–H···O$_{enolate}$ and C–H···N$_{azide}$ hydrogen bonds 38 into chains parallel with [100]. Further, the chains are connected by intermolecular hydrogen bonds that involve solvent methanol molecules serving as hydrogen bond donor (O2–H2A···N3) and hydrogen bond acceptor (C7–H7B···O2 and C9–H9C···O2) into layers parallel with (0–11) lattice plane (Table S5 and Fig. S2). The shortest separation of 4.6162(16) Å between the centers of gravity of the 1,3-thiazole rings is observed between the neighboring (0 −1 1) layers.

Crystal structure of complex 3. The asymmetric unit of 3 consists of the neutral [ZnL$^1$(N$_3$)$_2$] complex. The molecular structure of [ZnL$^1$(N$_3$)$_2$] with atom numbering scheme is shown in Fig. 3. Selected bond distances and valence angles are given in Table S3. In the [ZnL$^1$(N$_3$)$_2$] complex, the Zn(II) ion has fivefold coordination with NNO-set of donor atoms of L$^1$ and two nitrogen atoms (N5, N8) from azido ligands. The calculated $\tau$ value of 0.43 for [ZnL$^1$(N$_3$)$_2$] indicates that the five-coordination geometry of the Zn(II) ion is almost midway between square-based pyramidal and
trigonal bipyramidal form. The greatest basal angles N1-Zn1-O1 and N8-Zn1-N2 are 149.06(12)° and 123.30(18)°, respectively. In comparison with complex 3, the other structurally related five-coordinate Zn(II) complexes containing heteroaromatic hydrazones of Girard's T reagent and monodentate ligands (N₃⁻, NCO⁻, NCS⁻ or Cl⁻) exhibit a somewhat smaller degree of trigonal distortion from ideal square-based pyramidal configuration, as indicated by τ values being in the range 0.31–0.36 ⁴⁻, ³⁹–⁴¹. The dihedral angle of nearly 4.0° between two five-membered chelate rings (Zn–N–C–C–N and Zn–N–N–C–O) shows the non-coplanar nature of the metal-ligand system in 3. The Zn–Nₐr 2.209(3) Å and Zn–Nᵢmime 2.064(3) Å bond lengths observed in 3 fit into the range of values 2.206(6)–2.344(2) Å and 2.049(3)–2.088(6) Å, respectively, observed for the analogous Zn(II) complexes [ZnL¹(NCS)₂]·2H₂O ²⁷, [ZnL²(NCS)₂]·0.5MeOH ³⁹, [ZnL³(N₃)₂] ⁴¹, [ZnL³(NCO)₂] ⁴¹, and [ZnL³(N₃)₁.₆₅Cl₀.₃₅] ⁴⁰ (L³ = condensation product of 2-quinolinecarboxaldehyde and trimethylammoniumacetohydrazide chloride), while the Zn–Oₑnąlate bond is slightly longer 2.230(2) Å vs. 2.146(5)–2.222(2) Å. The azido ligands in 3 are coordinated to Zn(II) ion in bent mode, with Zn–N–N angles of 122.5(3) and 122.7(5)°. In the crystals of 3, the complex molecules [ZnL¹(N₃)₂] are self-assembled into supramolecular layers parallel with the (100) lattice plane through weak intermolecular C–H···N and C–H···O hydrogen bonds ³⁸ (Table S6 and Fig. S3).

![Fig. 1 ORTEP representation of the [Cu(L¹)(N₃)(CH₃OH)]⁺ complex cation in [CuL¹(N₃)(CH₃OH)]BF₄. Thermal ellipsoids are drawn at the 30% probability level.](image)
Fig. 2 ORTEP representation of the $[\text{Mn}_2\text{L}_1^2(\mu_{1,1}-\text{N}_3)_2(\text{N}_3)_2]$ in $[\text{Mn}_2\text{L}_1^2(\mu_{1,1}-\text{N}_3)_2(\text{N}_3)_2] \cdot 2\text{CH}_3\text{OH}$. Unlabelled part of the molecule is generated by symmetry operation $1-x$, $1-y$, $1-z$. Solvent CH$_3$OH molecules have been omitted for clarity. Thermal ellipsoids are drawn at the 30% probability level.
Fig. 3 ORTEP representation of the [ZnL₁(N₃)₂] complex. Thermal ellipsoids are drawn at the 30% probability level.

Computational results

DFT calculations were performed to elucidate the structures of Cu(II), Mn(II), and Zn(II) complexes in DMSO solution. Free energy changes, Δ_rG(298 K) of several probable reactions starting from the X-ray determined structures of 1, 2, and 3 were investigated. There is an excellent agreement between DFT optimized and X-ray structures for all three complexes (Fig. S13). Δ_rG(298 K) was calculated based on the difference in Gibbs free energies of products and reactants at ZORA-M06-2X/TZP-COSMO(DMSO)//ZORA-BP86-D3/TZP-COSMO(DMSO) level of theory. For the Cu(II) complex, we explored the formation of binuclear complex [Cu₂L₁(N₃)₂(CH₃OH)]²⁺, dissociation of weakly coordinated CH₃OH to form square-planar [CuL₁(N₃)]⁺, and several potential modes of coordination of DMSO to the Cu(II) center (reactions 1–5, Table 1). The results clearly show that the formation of square planar [CuL₁(N₃)]⁺ (Fig. 4) from 1 is thermodynamically favored. For Mn(II) complex, we investigated dissociation of crystal CH₃OH from 2 and dissociation of binuclear [Mn₂L₁(μ-1,1-N₃)₂(N₃)] forming mononuclear pentacoordinate complex [MnL₁(N₃)₂] (reactions 6 and 7, Table 1. Subsequent formation of octahedral Mn(II) complexes with a solvent molecule (DMSO) as the sixth ligand are also considered (reactions 8 and 9, Table 1). The results reveal that thermodynamically most favorable
is the formation of pentacoordinate $[\text{Mn}L^1(N_3)_2]$ complex (Fig. 5). However, according to the calculated $\Delta G$ of only $-1$ kcal/mol for reaction 7, binuclear $[\text{Mn}_2L^1_2(\mu-1,1-N_3)_2(N_3)_2]$ is also expected to be present in the DMSO solution. Two Mn(II) centers in $[\text{Mn}_2L^1_2(\mu-1,1-N_3)_2(N_3)_2]$ are weakly ferromagnetically coupled, with the exchange constant $J = 6.0 \text{ cm}^{-1}$ calculated in broken-symmetry DFT approach at ZORA-M06-2X-COSMO(DMSO)/TZP level of theory. Analogous reactions were considered for Zn(II) complex, i.e., formation of binuclear complex $[\text{Zn}_2L^1_2(\mu-1,1-N_3)_2(N_3)_2]$ (reaction 10, Table 1) and formation of six-coordinate complexes (reactions 11 and 12, Table 1). The calculations disclose that $[\text{Zn}L^1(N_3)_2]$ complex (complex 3) is thermodynamically preferred in DMSO solution.

### Table 1

Gibbs free energy changes ($\Delta G$ in kcal/mol at $T=298.15 \text{ K}$) calculated at ZORA-M06-2X/TZP-COSMO(DMSO)/ZORA-BP86-D3/TZP-COSMO(DMSO) level of theory for the formation of different Cu(II), Mn(II) and Zn(II) complexes starting from the X-ray determined structures of 1, 2 and 3.

| Reaction                                                                 | $\Delta G$ (298 K) |
|--------------------------------------------------------------------------|-------------------|
| 1. $2[\text{Cu}L^1(N_3)(\text{CH}_3\text{OH})]^+ \rightleftharpoons [\text{Cu}_2L^1_2(N_3)_2(\text{CH}_3\text{OH})_2]^{2+}$ | 8.90              |
| 2. $[\text{Cu}L^1(N_3)(\text{CH}_3\text{OH})]^+ \rightleftharpoons [\text{Cu}L^1(N_3)]^+ + \text{CH}_3\text{OH}$                  | -2.90             |
| 3. $[\text{Cu}L^1(N_3)(\text{CH}_3\text{OH})]^+ + \text{DMSO} \rightleftharpoons [\text{Cu}L^1(N_3)(\text{CH}_3\text{OH})(\text{DMSO})]^+$ | 4.58              |
| 4. $[\text{Cu}L^1(N_3)]^+ + \text{DMSO} \rightleftharpoons [\text{Cu}L^1(N_3)(\text{DMSO})]^+$                               | 2.96              |
| 5. $[\text{Cu}L^1(N_3)]^+ + 2\text{DMSO} \rightleftharpoons [\text{Cu}L^1(N_3)(\text{DMSO})_2]^+$                             | 7.33              |
| 6. $[\text{Mn}_2L^1_2(\mu-1,1-N_3)_2(N_3)_2] \cdot 2\text{CH}_3\text{OH} \rightleftharpoons [\text{Mn}_2L^1_2(\mu-1,1-N_3)_2(N_3)_2] + 2\text{CH}_3\text{OH}$ | -9.93             |
| 7. $[\text{Mn}_2L^1_2(\mu-1,1-N_3)_2(N_3)_2] \rightleftharpoons 2[\text{Mn}L^1(N_3)_2]$                                      | -1.07             |
| 8. $[\text{Mn}L^1(N_3)_2] + \text{DMSO} \rightleftharpoons \text{cis-[Mn}L^1(N_3)_2(\text{DMSO})]$                                 | 2.96              |
| 9. $[\text{Mn}L^1(N_3)_2] + \text{DMSO} \rightleftharpoons \text{trans-[Mn}L^1(N_3)_2(\text{DMSO})]$                            | 2.47              |
| 10. $2[\text{Zn}L^1(N_3)_2] \rightleftharpoons [\text{Zn}_2L^1_2(\mu-1,1-N_3)_2(N_3)_2]$                                         | 10.29             |
| 11. $[\text{Zn}L^1(N_3)_2] + \text{DMSO} \rightleftharpoons \text{cis-[Zn}L^1(N_3)_2(\text{DMSO})]$                             | 6.05              |
| 12. $[\text{Zn}L^1(N_3)_2] + \text{DMSO} \rightleftharpoons \text{trans-[Zn}L^1(N_3)_2(\text{DMSO})]$                           | 7.85              |
Antimicrobial activity

The antibacterial activity of the synthesized complexes, their precursors HLCl, NaN3 and appropriate salts was evaluated against a panel of five Gram-positive and five Gram-negative bacteria. The MIC data are given in Table 2. All three complexes showed antibacterial activity...
against all tested bacterial strains. For complexes 1 and 2, precursor compounds either do not have or show low antibacterial activity. The most active complex 2 is also the only binuclear complex in the series. Its activity towards *P. aeruginosa* is over twice lower than the activity of chloramphenicol, while against *P. hauseri*, the activity was four times lower than the control compound. Complex 1 displayed the best activity towards *E. coli* strain and very weak selectivity towards Gram-negative bacteria. The lowest antibacterial activity was obtained for complex 3. In some cases, its activity was lower than that of the parent salt. A comparison of antimicrobial activity of binuclear azido bridged complexes of Mn(II) (complex 2) and Ni(II) with the same ligand system, showed that complex 2 has higher antimicrobial activity. Even with this slightly lower activity, binuclear Ni(II) complex in most cases has higher activity than of mononuclear Cu(II) and Zn(II) complexes. Bearing in mind these two facts, the reason for higher antibacterial activity in the case of bimetallic Mn(II) complex can be explained by the existence of two metal centers.

The antifungal activity of the tested compounds is given in Table 3. All tested complexes showed a very good activity towards *A. braziliensis* and *S. cerevisiae*, and the strongest activity against these strains was displayed by binuclear complex 2. Its activity (MIC 0.09 mM) is comparable to the control compound amphotericin B. Against *C. albicans*, all three complexes showed moderate activity.

**Assessment of toxicity and radical scavenging activity**

The obtained results of the assessment of the toxicity compounds against freshly hatched nauplii *Artemia salina* as well as radical scavenging activity are given in Table 4. All synthesized complexes manifested moderate toxicity, compound 2 exhibiting the highest one. A possible interpretation of this result could be based on its good antibacterial activity. Since the nauplii live in symbiosis with some bacterial strains, it would be reasonable to assume that complex 2 exhibits its toxicity in this way.

Radical scavenging activity was determined by the DPPH test. Complex 1 showed the best activity. This is in line with the structure of the complexes. Namely, the central ion of complex 1 is redox-active Cu$^{2+}$. The radical scavenging activity of complex 1 is comparable to that of ascorbic acid.
Table 2 Antibacterial activity (MIC in mM)

| Compound                        | E. coli | P. aeruginosa | P. hauseri | K. pneumoniae | S. enterica | S. aureus | M. flavus | M. luteus | B. spizizenii | C. sporogenes |
|---------------------------------|---------|---------------|------------|---------------|-------------|-----------|-----------|-----------|---------------|--------------|
| HL Cl                            | /       | /             | /          | /             | /           | /         | /         | /         | /             | /            |
| 1                               | 1.19    | 2.37          | 4.74       | 2.37          | 4.74        | 4.74      | 4.74      | 4.74      | 4.74          | 4.74         |
| 2                               | 1.44    | 1.44          | 1.44       | 1.44          | 2.88        | 1.44      | 1.44      | 1.44      | 1.44          | 2.88         |
| 3                               | 2.75    | 2.75          | 5.50       | 2.75          | 2.75        | 2.75      | 2.75      | 2.75      | 5.50          | 5.50         |
| [NiL₂(μ-1,1-N₃),(N₃)₂]×4H₂O   | 1.56    | 3.12          | 3.12       | 3.12          | 1.56        | 3.12      | 1.56      | 1.56      | 3.12          | 3.12         |
| Cu(BF₄)₂×6H₂O                   | 7.33    | 7.33          | 7.33       | 7.33          | 7.33        | 7.33      | 7.33      | 7.33      | 7.33          | 7.33         |
| MnCl₂×4H₂O                      | 12.64   | 6.32          | 6.32       | 12.64         | 6.32        | 6.32      | 6.32      | 12.64     | 6.32          | 6.32         |
| Zn(BF₄)₂×6H₂O                   | 0.45    | 7.19          | 7.19       | 7.19          | 3.60        | 3.60      | 7.19      | 1.80      | 3.60          | 3.60         |
| NaN₃                            | 4.81    | 9.61          | /          | /             | 9.61        | 19.23     | 19.23     | 38.46     | 38.46         | /            |
| Chloramphenicol                 | 0.19    | 0.77          | 0.39       | 0.19          | 0.10        | 0.05      | 0.10      | 0.05      | 0.05          | 0.77         |
Table 3 Antifungal activity (MIC in mM)

|        | *A. braziliensis* | *C. albicans* | *S. cerevisiae* |
|--------|------------------|---------------|-----------------|
| HL Cl  | 0.07             | 0.14          | 4.48            |
| 1      | 0.30             | 2.37          | 0.30            |
| 2      | 0.09             | 1.44          | 0.09            |
| 3      | 0.17             | 0.69          | 0.17            |
| Cu(BF₄)₂·6H₂O | 3.67          | 7.33          | 3.67            |
| MnCl₂·4H₂O   | 6.32          | 12.64         | 6.32            |
| Zn(BF₄)₂·6H₂O | 3.60          | 3.60          | 3.60            |
| NaN₃     | 0.21             | 0.42          | 1.68            |
| Amphotericin B | 0.04          | 0.02          | 0.01            |

Table 4 Brine shrimp assay and DPPH radical scavenging activity

|            | LD50 (mM) | DPPH (mM) |
|------------|-----------|-----------|
| HL Cl      | 1.143     | 0.489     |
| 1          | 0.567     | 0.094     |
| 2          | 0.315     | 5.934     |
| 3          | 0.869     | 31.680    |
| Cu(BF₄)₂·6H₂O | 0.312     | 29.626    |
| MnCl₂·4H₂O   | 1.406     | /         |
| Zn(BF₄)₂·6H₂O | 0.884     | /         |
| NaN₃       | 0.537     | /         |
| K₂Cr₂O₇    | 0.077±0.016 | 0.079±0.018 |
| Ascorbic acid | /           | 0.079±0.018 |

Cytotoxic activities of Cu(II) and Mn(II)complexes

Examination of cytotoxic activities of Cu(II) complex (1), Mn(II) complex (2), Zn(II) complex (3) and their precursor compounds against human cancer cell lines and normal keratinocyte cell line is presented in Table 5. All three complexes showed concentration-dependent cytotoxic effects on tested cell lines. The Cu(II) complex (1) exerted the highest intensity of cytotoxic activity against melanoma A375, lung carcinoma A549, and prostate adenocarcinoma PC-3 cells with IC₅₀ values of 18.51 µM, 21.35, and 22.73 µM, respectively. The cytotoxicity of this complex was slightly lower against cervical adenocarcinoma HeLa and breast adenocarcinoma MCF7 cells (IC₅₀ values: 28.74 µM and 30.45 µM). A similar intensity of cytotoxic activity of 1 was observed against normal keratinocytes HaCaT with IC₅₀ value of 30.26 µM. Cu(II) complex (1) demonstrated stronger cytotoxicity against A375, A549, and PC-3 cancer cell lines compared to its cytotoxicity against normal HaCaT cells. The highest selectivity in the cytotoxic action of 1 was observed
against A375 melanoma cells compared to keratinocytes HaCaT (selectivity coefficient of 1.63). This complex exhibited notably higher cytotoxic activity against all tested cell lines when compared with the cytotoxic activity of its ligand and precursor compounds (Cu(BF$_4$)$_2$·6H$_2$O and NaN$_3$).

The Mn(II) complex (2) exerted the strongest cytotoxic effect on breast adenocarcinoma MCF7 cells with IC$_{50}$ value of 26.66 µM. The moderate cytotoxic activity of this complex was observed against HeLa, A549, A375, and PC-3 cancer cell lines (IC$_{50}$ values ranging from 40.92 to 52.07 µM). The examined Mn(II) complex (2) exerted moderate cytotoxicity against normal keratinocytes HaCaT with IC$_{50}$ value of 41.20 µM. The selectivity in the cytotoxic activity of this complex was shown only against breast cancer MCF7 cells compared with its activity against the normal cell line (selectivity coefficient of 1.55). The cytotoxic activities of its ligand and precursor compounds NaN$_3$ and MnCl$_2$·4H$_2$O were lower than the activity of 2. The only exception of this trend was slightly lower cytotoxicity of the complex 2 against A375 cells compared with the activity of salt MnCl$_2$·4H$_2$O against A375 cells.

The Cu(II) complex demonstrated stronger cytotoxic effects on tested cancer cell lines than Mn(II) complex. However, 2 showed selectivity to breast adenocarcinoma MCF7 cells in contrast to complex 1. The Zn(II) complex exerted the lowest cytotoxic activity against all tested cancer cell lines compared to Cu(II) and Mn(II) complexes including its precursor compounds.

**Table 5.** Cytotoxic activity of Cu(II),Mn(II) and Zn(II) complexes and their precursor compounds (IC$_{50}$ [µM] average±SD)

|             | HeLa     | A375     | A549     | PC-3     | MCF7     | HaCaT    |
|-------------|----------|----------|----------|----------|----------|----------|
| HL/Cl       | ≥200     | 155.65±5.95 | >200     | 197.67±3.29 | 199.23±1.09 | 180.00±4.66 |
| 1           | 28.74±2.93 | 18.51±2.20 | 21.35±0.29 | 22.73±0.52 | 30.45±3.53 | 30.26±4.07 |
| 2           | 40.92±1.30 | 52.07±2.93 | 44.45±2.66 | 50.25±0.36 | 26.66±3.16 | 41.20±1.70 |
| 3           | 289.10±4.11 | 292.63±14.65 | ≈400     | 362.43±2.96 | 375.46±10.87 | 206.92±9.79 |
| NaN$_3$     | >200     | 190.28±13.75 | >200     | >200     | >200     | >200     |
| Cu(BF$_4$)$_2$·6H$_2$O | 123.14±8.04 | 110.92±3.25 | 159.20±4.66 | 96.98±4.75 | 99.05±6.86 | 91.82±1.59 |
| MnCl$_2$·4H$_2$O | 48.68±1.25 | 46.07±5.55 | 153.86±4.33 | 118.94±19.10 | 60.50±3.48 | 79.56±1.21 |
| Zn(BF$_4$)$_2$·6H$_2$O | 171.06±2.85 | 131.85±11.88 | 199.22±1.10 | 192.77±2.63 | 198.78±1.72 | 114.15±8.75 |
| cisplatin   | 4.00±0.47 | 2.46±0.34 | 12.74±1.26 | 12.29±1.60 | 17.82±2.58 | 2.25±0.18 |

**Experimental**
Materials and methods

2-Acetylthiazole (99%) was obtained from Acros, Girard’s T reagent (99%) from Aldrich, DMSO-\(d_6\) from Merck, methanol from Betahem (Belgrade, Serbia), MnCl\(_2\)·4H\(_2\)O from Kemika d.d. (Zagreb, Croatia), NaN\(_3\) from Riedel-de Haën, Cu(BF\(_4\))\(_2\)·6H\(_2\)O and Zn(BF\(_4\))\(_2\)·6H\(_2\)O from Sigma-Aldrich. IR spectra were recorded on a Nicolet 6700 FT-IR spectrophotometer using the ATR technique in the region 4000–400 cm\(^{-1}\) (vs - very strong, s - strong, m - medium, w - weak). \(^1\)H and \(^{13}\)C NMR spectra were recorded on Bruker Avance 500 spectrometer (\(^1\)H at 500 MHz; \(^{13}\)C at 125 MHz) at room temperature using TMS as the internal standard in DMSO-\(d_6\). Chemical shifts are expressed in ppm (\(\delta\)) values and coupling constants (\(J\)) in Hz (splitting patterns: s - singlet, d - doublet). Elemental analyses (C, H, and N) were performed by standard micro-methods using the ELEMENTARVario ELIII C.H.N.S.O analyzer.

Synthesis

Synthesis of \(N,N,N\)-trimethyl-2-oxo-2-(2-(1-(thiazol-2-yl)ethylidene)hydrazinyl)ethan-1-aminium chloride (HL\(^1\)Cl) (\(E/Z = 1/1\)). The ligand HL\(^1\)Cl was synthesized by the reaction of Girard’s T reagent and 2-acetylthiazole according to the previously described method \(^{30}\).

IR (cm\(^{-1}\)): 3387 (w), 3128 (w), 3091 (m), 3017 (m), 2955 (s), 1701 (vs), 1612 (w), 1550 (vs), 1486 (s), 1401 (m), 1300 (w), 1201 (s), 1135 (w), 976 (w), 944 (w), 914 (m), 786 (m), 748 (w), 684 (w), 585 (w), 551 (w). (HL\(^1\)Cl -E). \(^1\)H NMR (500 MHz, DMSO-\(d_6\)), \(\delta\) (ppm): 2.41 (s, 3H, C5-H), 3.30 (s, 9H, C8-H), 4.60 (s, 2H, C7-H), 7.85 (d, 1H, \(J_{C2-H/C3-H} = 5.0\) Hz, C2-H), 7.93 (d, 1H, \(J_{C2-H/C3-H} = 5.0\) Hz, C3-H), 11.61 (s, 1H, N-H). \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)), \(\delta\) (ppm): 13.90 (C5), 53.65 (C8), 63.01 (C7), 123.33 (C2), 143.94 (C3), 146.98 (C4), 161.23 (C1), 167.04 (C6). (HL\(^1\)Cl -Z). \(^1\)H NMR (500 MHz, DMSO-\(d_6\)), \(\delta\) (ppm): 2.53 (s, 3H, C5-H), 3.34 (s, 9H, C8-H), 4.82 (s, 2H, C7-H), 7.85 (d, 1H, \(J_{C2-H/C3-H} = 5.0\) Hz, C2-H), 7.93 (d, 1H, \(J_{C2-H/C3-H} = 5.0\) Hz, C3-H), 11.86 (s, 1H, N-H). \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)), \(\delta\) (ppm): 15.05 (C5), 53.89 (C8), 63.76 (C7), 123.65 (C2), 143.97 (C3), 150.80 (C4), 166.78 (C1), 167.34 (C6).

Synthesis of complex [CuL\(^1\)(N\(_3\))(CH\(_3\)OH)]BF\(_4\) (1). The Cu(II) complex was synthesized by the reaction of HL\(^1\)Cl (70 mg, 0.25 mmol) and Cu(BF\(_4\))\(_2\)·6H\(_2\)O (86 mg, 0.25 mmol) in methanol (20 mL). After complete dissolution of Cu(BF\(_4\))\(_2\)·6H\(_2\)O in the reaction mixture, NaN\(_3\) (65 mg, 1 mmol) was added. The mixture was refluxed for 2 h. Green crystals were obtained after slow evaporation of the solvent in a refrigerator (\(\sim 4\) °C) for seven days. Yield: 42 mg (36 %). Elemental analysis
calcd. for $\text{C}_{11}\text{H}_{26}\text{BCuF}_{4}\text{N}_{7}\text{O}_{2}\text{S}$: C 28.43%, H 4.34%, N 21.10%, S 6.90%; found: C 28.53%, H 4.15%, N 21.17%, S 6.89%.

IR (cm$^{-1}$): 3352 (w), 3317 (w), 3077 (m), 3050 (s), 2970 (m), 2940 (w), 2047 (vs), 1829 (w), 1698 (w), 1604 (w), 1522 (s), 1477 (m), 1444 (m), 1413 (m), 1395 (m), 1325 (m), 1287 (m), 1239. (w), 1159 (w), 1124 (w), 1088 (w), 1053 (m), 1007 (m), 961 (w), 939 (w), 917 (m), 878 (w), 783 (m), 735 (w), 656 (w), 631 (w), 563 (w).

**Synthesis of complex $[\text{Mn}_{2}\text{L}_{12}(\mu-1,1-N_{3})_{2}(N_{3})_{2}]\cdot2\text{CH}_{3}\text{OH}$ (2).** The Mn(II) complex was synthesized by the reaction of $\text{HL}_{1}\text{Cl}$ (70 mg, 0.25 mmol) and $\text{MnCl}_{2} \cdot 4\text{H}_{2}\text{O}$ (50 mg, 0.25 mmol) in methanol (20 ml). After complete dissolution of Mn(II) salt, $\text{NaN}_{3}$ (65 mg, 1 mmol) was added. The mixture was stirred for 2 h at 60 °C. Orange crystals were obtained after slow evaporation of the solvent in a refrigerator (~ 4 °C) for 14 days. Yield: 50 mg (24 %). Elemental analysis calcd. for $\text{C}_{22}\text{H}_{40}\text{Mn}_{2}\text{N}_{20}\text{O}_{4}\text{S}_{2}$: C 32.12%, H 4.90%, N 34.05%, S 7.80%; found: C 31.95%, H 4.87%, N 34.15%, S 7.83%.

IR (cm$^{-1}$): 3388 (s), 3086 (m), 3036 (m), 2111 (w), 2042 (vs), 1688 (w), 1643 (w), 1595 (w), 1533 (s), 1481 (s), 1425 (m), 1331 (m), 1273 (m), 1202 (w), 1136 (w), 1115 (w), 1062 (w), 1043 (w), 1004 (w), 928 (w), 907 (w), 768 (w), 723 (w), 640 (w).

**Synthesis of complex $[\text{ZnL}_{1}(N_{3})_{2}]$ (3).** The Zn(II) complex was synthesized by the reaction of $\text{HL}_{1}\text{Cl}$ (70 mg, 0.25 mmol) and $\text{Zn(BF}_{4})_{2} \cdot 6\text{H}_{2}\text{O}$ (86 mg, 0.25 mmol) in methanol (20 ml). After complete dissolution of $\text{Zn(BF}_{4})_{2} \cdot 6\text{H}_{2}\text{O}$ in the reaction mixture, $\text{NaN}_{3}$ (65 mg, 1 mmol) was added. The mixture was refluxed for 2 h and filtered. Yellow crystals were obtained after slow evaporation of the solvent in a refrigerator (~ 4 °C) for seven days. Yield: 40 mg (41 %). Elemental analysis calcd. for $\text{C}_{10}\text{H}_{16}\text{N}_{10}\text{OSZn}$: C 30.82%, H 4.14%, N 35.94%, S 8.23%; found: C 30.76%, H 4.18%, N 35.83%, S 8.21%.

IR (cm$^{-1}$): 3378 (w), 3078 (w), 3054 (w), 3009 (w), 2964 (w), 2057 (vs), 1600 (w), 1540 (s), 1481 (w), 1433 (w), 1407 (w), 1339 (m), 1285 (w), 1203 (w), 1153 (w), 1116 (w), 1079 (w), 1057 (w), 1009 (w), 975 (w), 971 (w), 880 (w), 738 (w), 642 (w). $^1\text{H}$ NMR (500 MHz, DMSO-$d_6$), δ (ppm): 2.53 (s, 3H, C5-H), 3.23 (s, 9H, C8-H), 4.13 (s, 2H, C7-H), 7.92 (d, 1H, $J_{\text{C2-H/C3-H}} = 5.0$ Hz, C2-H), 8.04 (d, 1H, $J_{\text{C2-H/C3-H}} = 5.0$ Hz, C3-H). $^{13}\text{C}$ NMR (125 MHz, DMSO-$d_6$), δ (ppm): 15.73 (C5), 53.96 (C8), 67.11 (C7), 124.82 (C2), 143.44 (C3), 147.32 (C4), 165.93 (C1), 171.59 (C6).
X-ray crystallography

Crystal data and refinement parameters of compounds 1–3 are listed in Table 6. Single crystal X-ray diffraction data were collected at room temperature on an Agilent SuperNova dual-source diffractometer with an Atlas detector equipped with mirror-monochromated Mo–Kα radiation (λ = 0.71073 Å). Data processing was performed with CrysAlis PRO. The structures were solved with Olex software using SHELXT or SHELXS and refined by a full-matrix least-squares based on F² using SHELXL. All non-hydrogen atoms were refined anisotropically. All other hydrogen atoms were included in the model at geometrically calculated positions and refined using a riding model. The ORTEP-3 for Windows and MERCURY programs were used for graphical presentations. Crystallographic data for complexes 1–3 have been deposited with the Cambridge Crystallographic Data Centre as supplementary material CCDC 2110386–2110388. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Table 6 Crystal data and structure refinement details for 1, 2 and 3

|   | 1              | 2              | 3              |
|---|----------------|----------------|----------------|
| formula | C\textsubscript{11}H\textsubscript{20}BCuF\textsubscript{4}N\textsubscript{7}O\textsubscript{2}S | C\textsubscript{22}H\textsubscript{40}Mn\textsubscript{2}N\textsubscript{20}O\textsubscript{4}S\textsubscript{2} | C\textsubscript{10}H\textsubscript{16}N\textsubscript{10}OSZn |
| Fw (g mol\textsuperscript{-1}) | 464.75 | 822.74 | 389.76 |
| crystal size (mm) | 0.60×0.60×0.20 | 0.40×0.40×0.20 | 0.60×0.30×0.20 |
| crystal color | green | orange | yellow |
| crystal system | monoclinic | triclinic | monoclinic |
| space group | P 2\textsubscript{1}/n | P–1 | P 2\textsubscript{1}/c |
| a (Å) | 7.0033(3) | 9.6427(5) | 13.0826(10) |
| b (Å) | 10.8941(3) | 10.8396(5) | 10.2506(7) |
| c (Å) | 25.6059(9) | 10.8617(8) | 13.1685(13) |
| α(°) | 90 | 106.971(5) | 90 |
| β(°) | 97.242(4) | 103.497(5) | 111.237(10) |
| γ(°) | 90 | 112.469(5) | 90 |
| V(Å\textsuperscript{3}) | 1938.01(12) | 923.85(10) | 1646.0(3) |
| Z   | 4   | 1   | 4 |
|-----|-----|-----|---|
| calcd density (g cm$^{-3}$) | 1.593 | 1.479 | 1.573 |
| $F(000)$          | 948   | 426   | 800  |
| no. of collected reflns | 17559 | 10077 | 13910 |
| no. of independent reflns | 4425   | 4144   | 3779  |
| $R_{int}$ | 0.0235 | 0.0311 | 0.0315 |
| no. of reflns observed | 3789   | 3319   | 2489  |
| no. parameters | 287   | 232   | 212  |
| $R[I > 2\sigma (I)]$ | 0.0388 | 0.0371 | 0.0481 |
| $wR^2$(all data) | 0.1110 | 0.0982 | 0.1230 |
| Goof, $S$ | 1.157 | 1.072 | 1.084 |
| maximum/minimum residual electron density (e Å$^{-3}$) | +0.48/−0.34 | +0.28/−0.31 | +0.63/−0.77 |

$^a R = \sum|F_o| - |F_c|/\sum|F_o|$, $^bwR_2 = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]\}^{1/2}$, $^cS = \{\sum[(F_o^2 - F_c^2)^2]/(n/p)\}^{1/2}$ where $n$ is the number of reflections and $p$ is the total number of parameters refined.

### Computational Details

All DFT calculations were done with the ADF program package (version 2017)\(^{49-51}\). Relativistic effects were accounted for by the scalar-relativistic Zeroth-Order Regular Approximation (ZORA)\(^{52-54}\). The all-electron triple-zeta Slater-type orbitals plus one polarization function (TZP) basis set was used for all atoms. All open-shell systems are treated with unrestricted formalism in their high-spin state. The COSMO solvation model\(^{55, 56}\), as implemented in ADF\(^{57}\), with DMSO as solvent was used. Geometry optimizations were performed using general gradient functional consisting of Becke’s exchange\(^{58}\) and Perdew’s correlation\(^{59}\) with Grimme’s third-generation dispersion energy correction\(^{60}\) and Becke–Johnson damping\(^{61}\), i.e., BP86-D3. Cartesian coordinates of all optimized structures are available in Electronic Supplementary Information (ESI). Analytical harmonic frequencies\(^{62-64}\) were calculated at the same level of theory. All normal modes with small frequencies (<50 cm$^{-1}$) were rescanned numerically\(^{65, 66}\) to ascertain that all the optimized structures correspond to the minima on the potential energy surface. Vibrational analysis in the quasi-harmonic approximation as proposed by Truhlar\(^{67, 68}\) (frequency cut-off 100 cm$^{-1}$) has been
used to evaluate zero-point effects and entropic and thermal corrections to the Gibbs free energy at 298 K. Because vibrational analysis is done at a standard state of 1 atm, a conversion to 1 mol/dm$^3$ solution standard state is done. This gives a correction of 1.89 kcal/mol to the free energies (at 298 K). This correction is important only for reactions where the number of moles changes. In reactions where DMSO is involved, the free energy correction due to the conversion to the solvent standard state (13.98 mol/dm$^3$) equals 3.46 kcal/mol (at 298 K). Entropy correction due to the spin-multiplicity ($R\ln(g_s)$, $g_s$ is the spin-degeneracy of a complex, $R$ is the universal gas constant) is employed. When binuclear $[\text{Mn}_2\text{L}_1\text{L}_2(\mu_{-1,1}-\text{N}_3)_2(\text{N}_3)_2]$ is considered, low-lying excited states due to the exchange coupling are included in thermochemical analysis. Electronic energies used to calculate the Gibbs free energy were evaluated with M06-2X meta-hybrid functional, at ZORA-BP86-D3/TZP-COSMO(DMSO) geometries. LibXC library was used for M06-2X calculations. Free energy changes for each considered reaction were corrected for the basis set superposition error by fragment approach and “ghost atoms” in ADF. The exchange coupling constant $J$ in $[\text{Mn}_2\text{L}_1\text{L}_2(\mu_{-1,1}-\text{N}_3)_2(\text{N}_3)_2]$ was calculated with broken-symmetry DFT formalism at ZORA-M06-2X/TZP level of theory at X-ray and ZORA-BP86-D3/TZP-COSMO(DMSO) geometries according to the Yamaguchi approach. Broken symmetry solutions are obtained from the high-spin states with the spin-flip method. Calculated exchange coupling constants were used to estimate relative energies of low-lying spin-states through diagonalization of the spin Hamiltonian ($H = -2JS_1S_2$).

**Antimicrobial activity**

Antimicrobial activity was tested against a panel of microorganisms including: Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Proteus hauseri* (ATCC 13315), *Klebsiella pneumoniae* (ATCC 10031), *Salmonella enterica* subsp. *enterica* serovar Enteritidis (ATCC 13076), Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), *Bacillus spizizenii* (ATCC 6633), *Clostridium sporogenes* (ATCC 19404), *Micrococcus luteus* (ATCC 4698), *Micrococcus luteus* (ATCC 10240), yeasts *Candida albicans* (ATCC 10231), *Saccharomyces cerevisiae* (ATCC 9763) and fungal strain *Aspergillus brasiliensis* (ATCC 16404).

Antimicrobial activity was evaluated using broth microdilution method according to NCCLS [National Committee for Clinical Laboratory Standards, Approval Standard Document M7-A5, Villanova, Pa, USA, 2000]. The 96-well plates were prepared by dispensing 100 µl of
Mueller–Hinton broth for bacteria and Sabouraud dextrose broth for yeasts and fungi, into each well. A 100 µL aliquot from the stock solution of the tested compounds (concentration 10 mg/mL in DMSO) was added into the first row of the plate and double diluted by using a multichannel pipette. The direct colony method was used in the preparation of a suspension of bacteria and yeasts in sterile 0.9 % saline, while the process of preparing the suspension of fungal spores included gentle stripping of spore from agar slants with growing aspergilli into sterile 0.9 % saline. Suspension turbidity evaluation was conducted by comparison with 0.5 McFarland’s standard. A 10 µL of diluted bacterial, yeast or spores suspension was added to each well to give a final concentration of $5 \times 10^5$ CFU/mL for bacteria and $5 \times 10^3$ CFU/mL for fungi and yeast. Chloramphenicol served as a positive control for bacteria, while amphotericin B served as a positive control for yeasts and fungi.

The inoculated plates were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for the yeasts and fungi. The bacterial growth was visualized by adding 20 µL of 0.5% 2,3,5-triphenyltetrazolium chloride (TTC) aqueous solution. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the compounds that inhibited bacterial growth (red-colored pellet at the bottom of the wells after the addition of TTC).

**Brine shrimp assay**

About 20 g of commercially purchased lyophilized eggs of *Artemia salina* was added to 0.5 L of tap water, and air was passed through the suspension by a pump under illumination for 48 h. All tested compounds were dissolved in DMSO and various amounts (0.01–1 mg) were added to 950 µL of artificial seawater with freshly hatched nauplii. After 24 h illumination at room temperature, the number of dead and surviving nauplii were counted and statistically analyzed. LC$_{50}$ was defined as a concentration of compounds that caused the death of 50% of the nauplii. All samples were done in triplicate.

**DPPH radical scavenging activity**

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined by the method of Blois. Commercially available free radical DPPH was dissolved in methanol at concentration of $6.58 \times 10^{-5}$ M, while tested compounds were dissolved in DMSO. Into a 96-well microplate, 50 µL solutions of the tested compounds at concentrations range 10 to 0.02 mg/mL were loaded (50 µL DMSO in the control), and 100 µL of DPPH solution were added. After
incubation for 30 min at room temperature in the dark, the absorbance was measured at 517 nm. All the measurements were performed in triplicate and the scavenging activity of the tested derivatives was calculated as:

\[
\text{Scavenging activity (\%) = 100 \times \left( \frac{A_{\text{control}} - (A_{\text{sample}} - A_0)}{A_{\text{control}}} \right)}
\]

where \(A_{\text{control}}\) and \(A_{\text{sample}}\) refer to the absorbance of DPPH in control solution and sample, respectively, while \(A_0\) refers to the absorbance of the solutions of compounds, because of their color.

The IC\(_{50}\) was defined as the antioxidant concentration necessary to decrease the amount of the initial DPPH radical by 50 % and was calculated from the plotted graph of scavenging activities against the concentrations of the tested compounds. Ascorbic acid was employed as the positive control (concentrations from 50 to 500 \(\mu\)g mL\(^{-1}\)).

**Determination of cytotoxic activity**

The cytotoxic activity of newly synthesized Cu(II) and Mn(II) complexes and their precursor compounds was examined on five human cancer cell lines: cervical adenocarcinoma HeLa, melanoma A375, lung carcinoma A549, prostate adenocarcinoma PC-3, breast adenocarcinoma MCF7, as well as against normal human keratinocyte cell line HaCaT. Stock solutions of compounds were made in DMSO at concentration of 10 mM, with the exception of the stock solution of Cu(II)complex, which was made at concentration of 7.5 mM. The human cell lines were grown in complete nutrient medium RPMI-1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and penicillin-streptomycin solution. HeLa (3000 cells per well), A375 (3000 cells per well), A549 (5000 cells per well), MCF7 (7000 cells per well), PC-3 (5000 cells per well), and HaCaT cells (7000 cells per well) were seeded in 96-well microtiter plates and after 20 h the cells were treated with two complexes and their precursor compounds (five increasing concentrations were tested, ranging from 12.5 \(\mu\)M – 200 \(\mu\)M). Nutrient medium only was added to control cells. After 72 h treatment, the cell survival was determined by MTT assay according to the method firstly described by Mosmann 80, and modified by Ohno and Abe 81 and described in more detail elsewhere 82. Each of the three independent experiments was performed in triplicate. Chemotherapeutic drug cisplatin was used as a positive control.

**Conclusions**
The complexes 1–3 have been synthesized and characterized by X-ray crystallographic analysis, elemental analysis and IR spectroscopy. NMR spectroscopy results for Zn(II) complex showed its stability in solution. Hydrazone ligand is coordinated in a deprotonated form in all three complexes through the thiazole nitrogen, azomethine nitrogen, and carbonyl oxygen atoms. The five-coordination geometry of the Cu(II) ion (mononuclear complex 1) can be described as distorted square-based pyramidal, while in the case of Zn(II) ion (mononuclear complex 3) geometry is somewhere in-between square-based pyramidal and trigonal bipyramidal form. The geometry around Mn(II) ion (binuclear complex 2) is distorted trigonal prism with three donor atoms of hydrazone ligand, two nitrogen atoms from bridging azide anions, and one nitrogen atom from terminal azide anion. According to the DFT studies, Cu(II) complex is the most stable in square-planar [CuL¹(N₃)]⁺ geometry in DMSO solution, while in the same solution mixture of pentacoordinate [MnL¹(N₃)₂] and binuclear Mn(II) complex is predicted.

Novel Cu(II) complex showed pronounced cytotoxic effects against tested human cancer cell lines. The complex exerted higher intensity of cytotoxic activity against A375, A549, and PC-3 cancer cells compared to the intensity of cytotoxicity against normal keratinocytes HaCaT. In addition, a novel Mn(II) complex demonstrated potent cytotoxicity against MCF7 cells. Moderate cytotoxic activity of this complex was observed against other tested cancer cell lines. In general, the Cu (II) complex exhibits more potent cytotoxicity than the Mn(II) complex. However, the activity of complex 2 against breast cancer MCF7 line is very promising since it is only slightly weaker than the activity of cisplatin, but contrary to cisplatin, it is selective to the tumor cell line in comparison with the normal cell line (HaCaT).

**Conflicts of interest**

There are no conflicts to declare.

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