Metalo components exhibiting significant anticancer and antibacterial properties: a novel sandwich-type like polymeric structure

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Four new dicyanoargentate(I)-based complexes 1–4 were synthesized from certain metal ions with a tetratentate ligand \([N, N\text{-bis} (2\text{-hydroxyethyl})\text{-ethylenediamine}; N\text{-bishydeten}]\) and determined by diverse procedures (elemental, thermal, FT-IR, ESI–MS for 1–3 and, magnetic susceptibility and EPR for 1, and 2) including crystal analysis of 4. The crystal method revealed that complex 4 has a sandwich-type like polymeric chemical structure with layers formed by \([\text{Cd}(N-\text{bishydeten})_2]^{2+}\) cations and \([\text{Ag(CN)}_2]^-\) anions. The complexes were further characterized by fluorescence and UV spectroscopy to determine their physicochemical features. The complexes displayed a DNA binding activity within the same range as found for cisplatin, in addition to their strong stability in the presence of the physiological buffer system. The complexes were also investigated for pharmacological properties like interaction with DNA/Bovine serum albumin, anticancer and antibacterial activities. Physicochemical studies of DNA with the complexes suggested that the interaction mode between them are possibly both intercalative and groove binding types. These spectroscopic measurements also show that there may be a binding tendency between BSA and the complexes via hydrogen or Van der Waals bonds. The viability tests demonstrated that all the complexes exhibited antibacterial (1–4) and anticancer effects (2–4) toward ten diverse bacterial strains and three tumor cells (HT-29 colon adenocarcinoma, HeLa cervical cancer, and C6 glioma), respectively.

Despite the current advances in cancer therapy, the death rate from cancer and therapeutic agents are still increasing1. A significant number of studies have focused on the synthesis and design of a new antiproliferative agent to reduce the risk of drug resistance and cell toxicity2–4. Some of these studies are related to metallo compounds which constitute a significant part of agents with potent pharmacological activities, and their medicinal availability has been still explored5–6. For example, some macrocyclic polyamines containing Ni, Cu, and Ru could recognize TAR RNA molecules and cleave them, and affect the interaction of Tat-RNA7. Mn(II) complex of 2H-5-hydroxy-1,2,5-oxadiazo[3,4-f]1,10-phenanthroline has significant antitumor activity against HL-60, KB, Hela and BGC-823 cells. This compound binds with DNA by intercalating via the ligand \(L^8\). Mn(II) complex of 6,7-dicycanodipyridoquinoxaline intercalates into DNA base pairs via the ligand \(L\) and has significant antitumor...
**AgNO₃ + 2KCN → [Ag(CN)₂]⁻ + KNO₃**

\[ [Ag(CN)_2]^- + MCl₂ + N -bishydeten → [M(N -bishydeten)Ag_x(CN)y] \]

(M=Ni, Cu, Zn, Cd; x:y=1,2,3……)

**Figure 1. General reaction scheme of complex.**

Properties towards HL-60, KB, Hela and BGC-823 cells. This compound exhibit high antiproliferative effects within a µM range similar to those of antitumor drug 5-fluorouracil (5-FU)⁹. An another example of these metallo compounds with potent pharmacological activities is currently shown by cisplatin molecule and its analogs like carboplatin or oxaliplatin compounds, which have most widely prescribed metal-based anticancer drugs to treat a variety of cancer cases like lymphomas, lung, bladder, ovarian, and germ cell tumors¹⁰. 5-FU and cisplatin like carboplatin or oxaliplatin compounds, which have most widely prescribed metal-based anticancer drugs to treat a variety of cancer cases like lymphomas, lung, bladder, ovarian, and germ cell tumors. Among these, such coordination polymers attract considerable attention owing to their widespread usage or applications³⁷,⁴¹,⁴².

Cyanido metal complexes with d⁰ metal centers Ag(I), and Au(I) ions are two-coordinated structures in [Ag(CN)₃]⁻ and [Au(CN)₂]⁻ anionic forms having linear geometries. Both building blocks are ideal units to discover the utilize of argentophilicity or aurophilicity as a supramolecular design element of coordination polymer synthesis³⁵–³⁸. Among the cyanidometallates, Ag(I) polymers have high labiality of the Ag–donor bond coordination towards growth inhibition¹¹,¹². In subsequent studies, many metallo compounds against cancer were synthesized, characterized and tested to determine their pharmacological properties¹³–¹⁵. However, scientists are still in need for novel approaches to tackle the limitations of cancer treatment. Therefore, many research groups are trying to investigate new metallo-compounds with high efficacy and low toxicity, as an alternative to cisplatin. In this context, many silver or other metallo compounds with promising antitumor activity have been introduced¹⁶–²⁷. Among these, cyanido complexes are one of the alternatives that can be used in cancer therapy. Our studies showed that these complexes exhibit excellent anticancer, antibacterial and even antifungal activity²⁸–³⁴.

In this work, our group reported synthesis, structural characterization and some properties of polymeric [Ni(N-(bishydeten)Ag,(CN)₃)] (1), [Cu(N-(bishydeten)Ag(CN)] (2), [Zn(N-(bishydeten)Ag(CN)] (3) and [Cd(N-(bishydeten))̃[Ag(CN)] (4) along with the results of the X-ray structure assay of complex 4. Here, we focused on the analysis of the complex-DNA/BSA interaction together with their pharmacological activities like antibacterial (1–4), anticancer and cytotoxic properties (2–4) by using various powerful methods such as Lactate Dehydrogenase (LDH) Cytotoxicity and BrDU Cell ELISA assays. The action mechanisms of 2–4 were also explored by using DNA laddering, TUNEL, Topoisomerase I inhibitor activity, and cell migration assays. The results showed that complexes 2–4 were highly antiproliferative with low cytotoxic, and apoptotic characteristics. Furthermore, they suppressed Topoisomerase I activity and cell migration. Accordingly, we suggest that the complexes have a potential for use as novel anticancer drugs.

**Experimental section**

**Synthesis.** The synthesis acts were performed in the room temperature. The KCN (153 mg, 1.175 mmol) was added into a magnetically stirred solution of AgNO₃ (200 mg, 1.177 mmol) in ethyl alcohol (20 mL)/water (10 mL). Firstly, the Ni(II), Cu(II), Zn(II) and Cd(II) salts (1 mmol) were added to the clear solution of AgNO₃ in the alcohol, and it was stirred for about one hour. The resulting product was filtered, and also the filtrate obtained metal salt solution was added to the auxiliary ligand N-bishydeten (2 mmol, 0.296 g) solution prepared in the alcohol, and it was stirred for about one hour. The resulting product was filtered, and also the filtrate was left to crystallize under room conditions. Complexes 1–3 were obtained in low yields as powder crystals, while complex 4 also formed in low yields, but as single crystals (Table S1), (Fig. 1). The reason that complexes are obtained in low yields may be a consequence of the very high tendency of Ag(I) to produce stable complexes in the solution media or due to the steric hindrance around the coordination centre³⁶,³³–³⁴,⁴⁶–⁵¹.

**[Ni (N-(bishydeten)Ag,(CN)₃)] (1).** Pink precipitates were recorded with a yield of 43% for 1. Anal. Calc. for C₁₁H₁₆N₇O₂Ag₃Ni (%): C, 20.00; H, 2.44; N, 14.84 Found (%): C, 19.68; H, 2.14; N, 14.78. IR (KBr disk; cm⁻¹) 3596 υOH; 3336, 3280, 3119 υNH; 2979, 2904, 2861 υCH; 2163, 2129 υC≡N; 1452 δN–H; 1197 υCN; 1031 υCO. The effective magnetic moment, μeff (Bohr magnetons, μ₀); μeff (μₘr) values (μₛ=1; Magnetic moments with spin-orbital contributions) for 1 (Ni²⁺, d⁰): 4.52 (4.47)⁵². ESI-HR (m/z) [100%; M + 2H⁺] 658.49; analysis for 661.78.

**[Cu(N-(bishydeten)Ag(CN)] (2).** Light green precipitates were recorded with a yield of 40% for 2. Anal. Calc. for C₁₁H₁₆N₇O₂Ag₃Cu (%): C, 19.85; H, 2.42; N, 14.73 Found (%): C, 20.41; H, 2.91; N, 12.22. IR (KBr disk; cm⁻¹) 3081 υCH; 3313, 3235 υNH; 2915, 2869, 2846 υCH; 2134, 2125 υC≡N; 1473, 1450 δN–H; 1141 υCN; 1062 υCO. The effective magnetic moment, μeff (Bohr magnetons, μ₀); μeff (μₘr) values (μₛ=1; Magnetic moments with spin-orbital contributions) for 2 (Cu²⁺, d⁹): 2.17 (3.00)²⁷. ESI-HR (m/z) [100%; 2H + M⁺] 663.37; analysis for 2 (661.78).
"Experimental Section" and the IR spectra of the complexes and vibration frequency value shifts to a higher wavelength. As clearly indicated by the IR spectra of forms a bridge between the metal centers, the stretching vibration band of cyano usually splits as well, while the ′N–M≡N). When the cyano group coordinated with the metal (M–C≡N) or bridged between metal centers (M–C≡N–bishydeten)Ag3(CN)5] (3). Colorless precipitates were recorded with a 36% yield for 3. Anal. Calc. for C11H16N7O2Ag3Zn (%): C, 19.80; H, 2.42; N, 14.69 Found (%): C, 20.37; H, 2.70; N, 14.26. IR (KBr disk; cm−1) 3394 υOH; 3239, 3141 υNH; 2961, 2894, 2840 υCH; 2161, 2119 υC≡N; 1473, 1455 δN–H; 1114 υCN; 1079, 993 υC≡N.

Characterization of 1–4. The structures of complexes 1–4 were determined by elemental analysis, IR, EPR (for 1 and 2), ESI–MS (for 1–3) and X-ray crystallography (for 4) techniques, and the proposed molecular formulas were estimated by thermal analyses (DTA and TG/DTG) and magnetization measurement (for 1 and 2) techniques. Thermal analysis is like fingerprinting of materials, such that, each obtained thermal analysis curve is specific to the tested specimen, provided that a correct structure is proposed. For instance, the mass loss indicated by the TG curve of a synthesized compound can be interpreted correctly only if a correct molecular formula is introduced. Besides, the experimental effective magnetic moment (μeff) of a complex is consistent with the theoretical effective magnetic moment (μtheor) to the extent that a correct molecular formula makes the calculation. On the other hand, the typical peaks appearing in the ESI–MS spectra of 1–3 are attributed to [M + 2H]+. According to the characterization results, complexes 1–3 may have a molecular structure as given in Figure S1.

Results and discussion
IR spectra. The characteristic bands of the functional groups of all the complexes are presented in the "Experimental Section" and the IR spectra of the complexes and N-bishydeten ligand are depicted in Figure S2 (Supplementary Material). The most significant vibration frequency for cyanido complexes is known to be the CO. The images in Figs. 2 and 3, s5, s6, s7, and s8 were generated by using K. Brandenburg, Diamond-Crystal and Molecular Structure Visualization, Crystal Impact GbR, Vers. 4.5.2, Bonn, Germany, 2018.
On the other hand, the characteristic vibration frequency bands of the neutral ligand N-bishydeten in 1–4 complexes can be seen as another important evidence of the formation of the expected structures (Figure S2).

**Thermal analyses.** Thermogravimetric/thermogravimetric derivative–differential thermal analysis (TG/DTG–DTA) measurements of 1–4 also support the crystal composition (4) and the proposed structures (1–3) as shown in Figures S3 and S4. The TG/DTG curves of 1–4 are followed by a process in which a multi-step weight loss is observed from 35 to 1050 °C. The sharp peak at 300–400 °C in the thermal decomposition graph of complex 1 corresponds to an N-bishydeten ligand and two cyanide groups, while complex 2 corresponds only to the degradation of the N-bishydeten ligand at 500 °C (Figure S4). The neutral N-bishydeten ligand is degraded in the initial steps of the thermal decomposition which is followed by the thermal degradation of the cyanido ligand.

**Figure 3.** The sandwich-type like the structure of complex 4. H atoms were omitted, and C, N and O atoms were made invisible. (The image in this figure was generated by using K. Brandenburg, Diamond-Crystal and Molecular Structure Visualization, Crystal Impact GbR, Vers. 4.5.2, Bonn, Germany, 2018.)

**Table 1.** Hydrogen bonds (Å, o) for 4.

|          | d(D–H) | d(H⋯A) | d(D⋯A) | <(DHA) | Symmetry codes          |
|----------|--------|--------|--------|--------|-------------------------|
| N(2A)–H(2A)⋯N(6) | 0.90   | 2.62   | 3.407  | 145.87 | −x+1, −y+1, −z+1        |
| N(2A)–H(2A)⋯N(14) | 0.90   | 2.15   | 2.994  | 155.44 | −x+1, −y+1, −z+1        |
| O(1A)–H(1A)⋯N(14) | 0.82   | 2.52   | 3.130  | 132.30 | x+1, −y+3/2, z+1/2      |
| O(2A)–H(2A)⋯N(12) | 0.82   | 2.07   | 2.880  | 168.63 | −x+1, y+1/2, −z+1/2     |
| N(2B)–H(2B)⋯N(14) | 0.90   | 2.09   | 2.970  | 164.05 | −x+1, −y+1, −z+1        |
| O(1B)–H(1B)⋯N(14) | 0.82   | 2.37   | 2.996  | 132.96 | x+1, −y+3/2, z+1/2      |
| O(2B)–H(2B)⋯N(12) | 0.82   | 2.05   | 2.820  | 155.79 | −x+1, y+1/2, −z+1/2     |
| O(3A)–H(3A)⋯N(6)  | 0.82   | 2.00   | 2.773  | 156.27 | −                        |
| O(4A)–H(4A)⋯N(5)  | 0.82   | 2.16   | 2.977  | 171.23 | −                        |
| N(9A)–H(9A)⋯N(5)  | 0.90   | 2.44   | 3.243  | 148.69 | −x+1, y−1/2, −z+1/2     |
| N(9A)–H(9A)⋯N(12) | 0.90   | 2.60   | 3.340  | 140.07 | −                        |
| O(3B)–H(3B)⋯N(6)  | 0.82   | 1.87   | 2.650  | 156.36 | −                        |
| O(4B)–H(4B)⋯N(5)  | 0.82   | 2.27   | 2.049  | 157.70 | −                        |
| N(9B)–H(9B)⋯N(5)  | 0.90   | 2.13   | 3.021  | 170.44 | −x+1, y−1/2, −z+1/2     |
| N(9B)–H(9B)⋯N(12) | 0.90   | 2.57   | 3.369  | 148.07 | −                        |
Scheme 1. The molecular structure of N-bishydeten (ChemDraw Ultra 12.0).

Finally, the final stage of the thermal decomposition is the temperature at which the inorganic components corresponding to the metal residues are located. Experimental data indicate that the mass remaining in the thermal degradation for complexes 1–4 at 1050 °C is the weight corresponding to the inorganic components consisting of Ni + 3Ag (calc.: 57.87; found: 58.82), Cu + 3Ag (calc.: 58.18; found: 57.46), Zn + 3Ag (calc.: 58.29; found: 58.95) and Cd + 10Ag (calc.: 45.99; found: 45.77), respectively.

The crystal structure of 4. X-ray analysis revealed that 4 consists of an asymmetric unit, –CN–Cd1(N-bishydeten)–NC–Ag1–CN–Cd2(N-bishydeten)–NC–Ag4–CN–Cd1(N-bishydeten)–NC–Ag2–CN–Cd2(N-bishydeten)–NC–, at a three-dimensional zigzag chain structure similar to the ‘M’ shape (Fig. 2 and Figure S5; Supplementary Material). In the polymer chains like the structure of complex 4, the dicyano silver moieties were adopted slightly bent like conformer using intramolecular argentophilic interaction (Ag1…Ag2…A3 and Ag4…Ag5…Ag4) (Fig. 2 and Figure S6; Supplementary Material). In the structure, argentophilic interactions cooperatively act with HBs interaction (Table 1) which results in a stable macromolecular clustered structure.

The macromolecular structure is composed of a mixture of Cd–Ag sandwich-type like Cd–N, Cd–O and Ag–N clusters, in which the six coordinated \([\{\text{Cd}(N\text{-bishydeten})(\mu-N\text<decimal{5}))\}]^8\) cationic belts are sandwiched between anionic slices \([\{\text{Ag}({\mu-CN\text<decimal{2})})\}_{6}\{\text{Ag}(CN)\}_{3}\}]^6\) (Fig. 3 and Figures S7, S8; Supplementary Material). The centroid to centroid distance between each repeating cationic and anionic junction is 5.001 Å (Figure S7; Supplementary Material). The six-coordinated \([\{\text{Cd}(N\text{-bishydeten})(\mu-N\text<decimal{5}))\}]^8\) cationic belts contain Cd1 and Cd2 metal centers which are located in different planes and surrounded by the 2O- and 2N-atoms of tetradentate N-bishydeten ligand and the 4N-atoms of bridged dicyanoargentate anions (Ag1 and Ag4) (Fig. 2). The four Cd–O and four Cd–N distances for coordinated two N-bishydeten ligands to the Cd1 and Cd2 centers (Table S2; Supplementary Material) are 2.451(11) (Cd1–O1A), 2.392(11) (Cd1–O2B) and 2.429(18) (Cd2–O3A), 2.559(16) Å (Cd2–O4B) and 2.341(4) (Cd1–N2), 2.306(4) Å (Cd2–N7), 2.335(4) Å (Cd2–N7A) and 2.332(4) Å (Cd2–N11), 2.308(4) Å (Cd2–N11A) and 2.299(4) Å (Cd2–N7) and 2.299(4) Å (Cd2–N7). The significant differences between the Cd–O and Cd–N bond lengths can be attributed to the zigzag chain structure of M-shape formed in different planes (Figure S5). On the other hand, All the N–Cd–N, C–N–Cd and C–Ag–C which deviates remarkably from the 90° and 180°, which were likely the outcome of the steric limitations arising from the form of the ligands (Scheme 1). The N2A–Cd1–N1, O2A–Cd1–O1A, N9A–Cd2–N8 and O4B–Cd2–O3B angles formed by Cd1 and Cd2 centers is 170.0(4)°, 158.4(2)° and 165.6(4)°, respectively, and Cd2 centers–N-bishydetenCd–N distances for coordinated two anionic complexes reveal that EPR signals cannot be obtained at room temperature, but very low-intensity peaks can be seen at very low temperatures.

EPR and magnetic properties. The powder EPR spectra of complex 1 containing Ag⁺ and Ni²⁺ ions at room temperature could not be observed. This situation may be because Ag⁺ ion is diamagnetic and Ni²⁺ ion does not signal because it has short relaxation times at room temperature. EPR spectrum analyzes of Ni²⁺ ion-containing complexes reveal that EPR signals cannot be obtained at room temperature, but very low-intensity peaks can be seen at very low temperatures.

The powder EPR spectra of complex 2 are seen in Figure S9 in the Supplementary Material. The EPR spectra of 2 have been observed in the parallel and perpendicular components. The parallel peak is because the dc field is equal to the symmetry axis of the paramagnetic center. The values of \(g_\perp\) and \(g_{\parallel}\) extracted from the powder
spectrum of complex 2 are $g_\// = 2.210$, $g_\perp = 2.095$, respectively. This spectrum belongs to Cu$^{2+}$ ion ($S = 1/2$, $I = 3/2$). It can be inferred from the order of $g_\// > g_\perp > g_e$ ($g_e = 2.0023$, free electron $g$ value) that Cu$^{2+}$ is located in distorted items ($D_{4h}$) elongated along the ground state of the paramagnetic electron is $d_{2-\gamma^2}$ ($^2B_1g$ state) and $z$-axis$^{61-64}$. 

When the Lande $g$ values of Cu$^{2+}$ complexes containing tetracyanidometallate having neutral ligands are compared with those of complex 2, it is noticed that $g$ amounts are $g_\// > g_\perp > g_e$ $^{33,34,48,49,65-68}$. 

The magnetic susceptibilities of 1 and 2 were recorded in the temperature of 10–300 K. The temperature dependence of magnetic ($\chi_m$) and $\chi_mT$ are seen in Figures S10 and S11 (Supplementary Material) for both complexes. The variable temperature dependence of $\chi_m$ for both complexes were coordinated by the relation $\alpha + C/(T - \theta)$, which $\alpha$ is the temperature independent susceptibility (TIP)$^{69}$. For 2, the determined results are: $C = 0.588 \pm 0.0003$ emuK/mol Oe, $\alpha = 0.00027 \pm 0.000003$ emu/mol Oe and $-4.9 \pm 0.009$ K. As for 1, the determined fitting results: $C = 2.56 \pm 0.0005$ emu/mol Oe, $\alpha = 0.00077 \pm 0.000004$ emu/mol Oe and $= -0.6 \pm 0.002$ K. The good magnetic moment for 1, $\mu_{eff}$, was determined as 4.52 in Bohr magneton ($\mu_B$)$^{70}$. In this part, 10 K, for 1 and 2 could be very tiny antiferromagnetic interplay in the chemical structure, as observed in the insertion of Figures S10 and S11.

DNA topoisomerase I, DNA restriction endonucleases, and DNA binding studies. Determination of DNA topoisomerase I enzyme inhibitory activities. DNA topoisomerase that is an important target for anticancer agents are nuclear enzymes and alter the topological state of DNA molecule during the cell division and another cellular process such as replication and transcription$^{73,74}$. Today, some topoisomerase inhibitor compounds like Camptosar, topotecan, and irinotecan have been utilized in clinical practice. Hence, to figure out the antiproliferative activities of these molecules, for instance Camptothecin (Fig. 4), inhibited the DNA relaxation activity of topoisomerase I that they can be used as a new topoisomerase I inhibitor which acts through binding to topoisomerase I. The results of other studies also revealed that metal complexes bind to topoisomerase I and inhibited it$^{70,75,76}$.

Determination of DNA restriction endonucleases activity. In the presence of the 3 and 4, DNA digestion was complete, and two bands were observed near the well at the top of the lanes (Lanes 1 and 2). Treatment of KpnI and BamHI with 3 and 4 failed to inhibit the restriction endonucleases activity of these enzymes. These results indicated that 3 and 4 did not bind to pTOLT plasmid DNA. However, the 2 caused the formation of two DNA

Figure 4. A DNA unwinding assay was performed with 2U TOP1, 250 ng pHOT-1 supercoiled DNA, and IC$_{50}$ concentrations of 2, 3 and 4. The phases of the DNA molecule are denoted as II (Relaxed DNA), I (Nicked DNA), and, III (Supercoiled DNA). Lane 1 in this work is the supercoiled marker DNA; Also, Lane 2 represents the relaxed marker DNA molecule; Lane 3 in this study represents the negative standard (TOP1 + Supercoiled DNA); Finally, Lane 4 is the normal control (Camptothecin + TOP1 + Supercoiled DNA) and Lane 5–7 for this part represent test molecules over an IC$_{50}$ value-concentration.
bands (Lane 3) corresponding to the supercoiled and nicked DNA, that was observed in the undigested DNA (Lane 5) (Figure S12; Supplementary Material).

**DNA binding study.** UV-visible absorption spectroscopy technique has been utilized to work the interactions among DNA and 1–4. The type of test molecule–DNA interactions and the binding constant ($K_b$) may be evaluated by comparison of electronic spectra properties of the free test molecule and test molecule–DNA adduct. The binding constant of 1–4 with DNA can be obtained according to Wolfe–Shimmer equation, 

$$\frac{[\text{DNA}]}{(\varepsilon_a - \varepsilon_f)} = \frac{[\text{DNA}]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)}.$$  

The $K_b$ can be recorded from the ratio of the slope to intercept in the plot of [DNA] versus [DNA]/($\varepsilon_a - \varepsilon_f$), where $\varepsilon_f$ and $\varepsilon_a$ are the absorption coefficients of the 1–4 and its adduct, respectively. Figure 5 represents the interaction of 1–4 with CT-DNA. The inset graph has the plot of [DNA]/($\varepsilon_a - \varepsilon_f$) data which yielded the binding constant ($K_b$) of $2.3 \times 10^4 \text{ M}^{-1}$ for 1, $5.0 \pm 0.24 \times 10^4 \text{ M}^{-1}$ for 2, $2.7 \pm 0.19 \times 10^4 \text{ M}^{-1}$ for 3, and $4.6 \pm 0.21 \times 10^4 \text{ M}^{-1}$ for 4 (Fig. 5). There was a hyperchromic effect at the absorption bands of 2 and 4 indicating a strong interaction between them and DNA. However, the hypochromic effect was observed by the addition of increasing amounts of CT-DNA to 1 and 3. The hypochromic effect of 1 and 3 contributed to the intercalation of 1 and 3 into the DNA base pairs and can be explained by decreasing the distance between DNA bases and intercalated 1 or 3. Overall, the observed binding

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**Figure 5.** UV absorption spectra of 25 µM 1, 2, 3 and 4 in the absence (a) and presence of 6.25 µM (b), 12.5 µM (c), 25 µM (d), 50 µM (e), 100 µM (f), 200 µM (g), 800 µM (i) DNA.
values of 1 in measuring of the compounds were found to be linear in the scanning concentration range, and the linearity equation, which provides more evidence about the interaction between % RSD < 2% for the molecules. These molecules remained fairly stable (Figure S14 Table 2). The plots proved to have the ability of high solution stability in a buffer (Table 2). The molecules in physiological buffer (Phosphate buffered saline, 0.1 M, pH 7.4) were performed at regular intervals for 24 h. There were no changes in absorbance up to 24 h in complexes. Thus, the silver compounds proved to have the ability of high solution stability in a buffer (Table 2).

The results of these molecules were conducted by utilizing a simple spectrophotometric assay. The molecules in physiological buffer (Phosphate buffered saline, 0.1 M, pH 7.4) were performed at regular intervals for 24 h. There were no changes in absorbance up to 24 h in complexes. Thus, the silver compounds proved to have the ability of high solution stability in a buffer (Table 2).

This study was assessed using the absorbance values of eight diverse concentrations of the molecules within the same day and between various days. The repeatability, inner- and intra-day precision of the work displayed proved to have the ability of high solution stability in a buffer (Table 2).

### Stability study.

The results of these molecules were conducted by utilizing a simple spectrophotometric assay. The molecules in physiological buffer (Phosphate buffered saline, 0.1 M, pH 7.4) were performed at regular intervals for 24 h. There were no changes in absorbance up to 24 h in complexes. Thus, the silver compounds proved to have the ability of high solution stability in a buffer (Table 2).

### Antiproliferative actions of the Ag(I) molecules.

The antiproliferative activities of Ag(I) molecules and the starting molecules of the parent molecules, N-bishydeten and [Ag(CN)₂]⁻ compound, on cells, were monitored utilizing by the BrdU Cell Proliferation Assay (BCPA) (Fig. 6). To determine whether selectively killed the cancer agents in the absence of being detrimental to the standard cells, we determined the antiproliferative effects towards cancer with the same administrative dose (data not shown). Antiproliferative activities of complex 2 were higher on HT29 (0.87 ± 0.09 µM) and C6 (0.95 ± 0.09 µM) cells in comparison to 5FU or cisplatin (9–11%) tested and also were found to be moderately cytotoxic against HT29 cells. How-

| Parameters          | 1    | 2    | 3    | 4    |
|---------------------|------|------|------|------|
| Linearity range     | 1.95–250 | 1.95–250 | 1.95–250 | 1.95–250 |
| Linearity           | 0.98 | 0.96 | 0.95 | 0.97 |
| Accuracy, % RSD < 2% | 127.77 | 116.91 | 93.06 | 91.82 |
| Precision, % RSD < 2% | 0.95 | 0.97 | 0.98 | 0.98 |
| LOD                 | 18.27 | 33.57 | 40.34 | 27.03 |
| LOQ                 | 55.38 | 101.75 | 122.24 | 81.93 |
| % Error             | 12.00 | 11.47 | 19.83 | 9.17 |
| Linearity range     | 1.95–250 | 1.95–250 | 1.95–250 | 1.95–250 |

**Table 2.** UV–Vis spectrophotometric method.

| Parameters              | 1    | 2    | 3    | 4    |
|-------------------------|------|------|------|------|
| Linearity               | 0.95 | 0.97 | 0.98 | 0.98 |
| Accuracy, % RSD < 2%    | 127.77 | 116.91 | 93.06 | 91.82 |
| Precision, % RSD < 2%   | 0.95 | 0.97 | 0.98 | 0.98 |
| LOD                     | 18.27 | 33.57 | 40.34 | 27.03 |
| LOQ                     | 55.38 | 101.75 | 122.24 | 81.93 |
| % Error                 | 12.00 | 11.47 | 19.83 | 9.17 |

constant of the complexes against DNA and 5FU where binding constant were reported to be 5.73 × 10⁴ M⁻¹ and 9.7 × 10⁴ M⁻¹, respectively.59,60.

In addition to UV–Visible absorption spectroscopy technique, the ethidium bromide exchange studies were also conducted to determine the binding affinity between 2, 3, 4 and DNA. The emission spectrum data EB bound to DNA in the presence and absence of 2, 3, and 4 are depicted in Figure S13 (Supplementary Material). The decreases in the fluorescence intensity of EB-DNA in the presence of 2, 3, and 4 implied that they might intercalate into a pair of the DNA. The quenching of EB to CT-DNA by the 2 is in harmony with the Stern–Volmer equation, which provides more evidence about the interaction between 2 and DNA, and is shown in Figure S13. The Ksv value for the complex 2, 3, and 4 are 5.0 ± 0.32 × 10⁵ M⁻¹, 13.7 ± 0.41 × 10³ M⁻¹, 1.1 ± 0.12 × 10³ M⁻¹, respectively. The reaction of the 3 with CT-DNA is more powerful than those of the 2 and 4 complexes.

Stability study. The results of these molecules were conducted by utilizing a simple spectrophotometric assay. The molecules in physiological buffer (Phosphate buffered saline, 0.1 M, pH 7.4) were performed at regular intervals for 24 h. There were no changes in absorbance up to 24 h in complexes. Thus, the silver compounds proved to have the ability of high solution stability in a buffer (Table 2).

The find tumor specificity index results divided by the sum of the IC₅₀ values from normal cells (Vero) to the sum of the IC₅₀ values of the cancer cells (C6, HeLa, HT29) (Table 3). Molecule 2 recorded the best selectivity for the HT29 (3.23 TSI) and C6 (2.96 TSI) cells over the Vero cells while compounds 3 and 4 (1.29 TSI) displayed poor selectivity for the HT29 cells. The cell proliferation results disclosed that the Ag(I) molecules exhibited the best selectivity towards cancer cells.

Cytotoxic profile of the Ag(I) compounds. LDH test results revealed that 2, 3, and 4 exhibited the identical cytotoxic effects as the 5FU. Indeed, bridging ligand [Ag(CN)₂]⁻, caused greater cytotoxicity than positive control on some cell lines (Figure S15; Supplementary Material). It is observed that [Ag(CN)₂]⁻ is a highly toxic molecule towards both standard and tumorigenic cells. However, it was found to have a limited effect while examining the contribution of [Ag(CN)₂]⁻ to the antiproliferative and cytotoxic activities of our compounds. As seen in Figure 6 and Figure S15, the antiproliferative and cytotoxic activity of 2, 3, and 4 were lower than bridging ligand, recording that the cytotoxicity of [Ag(CN)₂]⁻ reduced to safe levels in Ag(I) compounds. All compounds except for the starting molecules of the parent molecules, [Ag(CN)₂]⁻ and [Ag(CN)₂]⁻ compound, on cells, were lower than bridging ligand, recording that the cytotoxicity of [Ag(CN)₂]⁻ reduced to safe levels in Ag(I) compounds. All compounds and 5FU or cisplatin (9–11%) tested and also were found to be moderately cytotoxic against HT29 cells. How-
However, an important reduction in cytotoxicity was obtained for 2 when their activity on cells was evaluated. The IC50 values obtained from our compounds (0.48–3.64 μM) were lower than those of 5FU (275.68–258.46 μM) or those of cisplatin (152.57–234.83 μM) (Table 3). However, it is necessary to conduct in vivo studies in order to determine the real cytotoxic effect of these compounds. An ideal anticancer drug would exterminate cancer cells without disturbing normal cells and has cytostatic profiles that can activate apoptosis.

Determination of the apoptotic effect of the Ag(I) complexes by DNA laddering method. DNA laddering revealed the 2, 3, and 4 induced the organization of DNA fragmentation in cancer cells in comparison to the standard cells (Fig. 7). Here, appearances of apoptotic morphology and DNA fragmentation may be a result of the activation of the extrinsic apoptotic pathways, including Ca2+ dependent endonucleases. Apoptosis assay is determined by controlling cell death which included cleavage of DNA molecule into regular fragments. In this part, we observed that our molecules could act through containing apoptosis on some cells. More studies were conducted to obtain the antiproliferative and apoptotic potentials of Ag molecules which are consistent with this work.

Table 3. IC50 values and tumor specificity index. *Values are given as the mean ± SD of three experiments and r² = 0.91 to 0.98. Significant at P < 0.05.

| Compounds | IC50 (µM) | Tumor specificity index |
|-----------|-----------|-------------------------|
|           | HeLa* | HT29* | C6* | Vero* | HeLa | HT29 | C6 |
| 2         | 3.64 ± 0.42 | 0.87 ± 0.09 | 0.95 ± 0.09 | 2.81 ± 0.32 | 0.77 | 3.23 | 2.96 |
| 3         | 3.19 ± 0.37 | 2.37 ± 0.32 | 3.34 ± 0.34 | 3.06 ± 0.35 | 0.96 | 1.29 | 0.92 |
| 4         | 0.61 ± 0.09 | 0.48 ± 0.08 | 0.60 ± 0.09 | 0.63 ± 0.09 | 1.03 | 1.31 | 1.05 |
| 5FU       | 275.68 ± 258.46 | 217.48 ± 19 | 258.46 ± 21 | 0.94 | 1.00 | 1.19 |
| Cisplatin | 230.41 ± 15 | 152.57 ± 14 | 207.51 ± 23 | 234.83 ± 19 | 1.02 | 1.54 | 1.13 |
| [Ag(CN)2]¯ | 5.13 ± 0.87 | 5.18 ± 0.93 | 5.08 ± 0.89 | 5.63 ± 0.96 | 1.10 | 1.09 | 1.11 |

Figure 6. Effects of 2, 3, 4 and [Ag(CN)2] on the proliferation of Vero cells, HeLa, C6, and HT-29. The growing cells were incubated with 2, 3, 4 and [Ag(CN)2] and the cell multiplication was obtained by the BrdU Elisa method.
Inhibitory concentration (IC20) allowed the suppression of cell migration without damaging the cell membrane with low cytotoxicity (data not shown). In addition, this cytotoxic ability of Ag(I) compounds at 20% maximal apoptotic agent against HT29 cells, exhibit TUNEL positive activity15,19,35. In a similar study performed with good-soluble Ag(I) compounds containing different ligand and Ni, Cu, Zn, and Cd metal salts complex acting as containing different ligands73,74,78,80,81,83–87.

The effect of the Ag(I) complexes in the morphology of the cells. As shown in Figure S17 (Supplementary Material), obvious morphological changes were recorded in the treated cells when in comparison to the untreated cells. Each cell line exposed to the compounds exhibited cytoplasmic blebs, shrinkage, anomalous...
globular structure; these are all hallmarks of the apoptotic cell death. The results in Figure S17 have also displayed the normal structure of the most control cells. Most of the treated cells had an abnormal fibroblast-like appearance and were detached from the plate surface. Moreover, the cells began to separate from one another and to appear smaller. These situations were consistent with the outcomes of TUNEL methods, and this finding was similar to those of previous studies. According to information found in the literature, the appearance of the cells treated with clearly indicated the quality and the number of cells in the flask monolayer were reduced.

**IHC investigation of slides treated by Ag(I) molecules.** Immunohistochemistry staining was found to reduce the expression of Bcl-2 and increase the expression of P53 in Ag(I) complexes-treated the cells, which emphasizes the apoptotic effects of these molecules (Figures S18 and S19). These findings are agreeable with those of similar works. The results also revealed that Ag(I) complexes treated cells significantly reduced the expression of cytokeratins (CK20 and CK7) releasing from proliferating or apoptotic cells. This condition can be associated with the reduced metastatic capability via an anti-migratory potential of these molecules due to the influenced intermediate filament (IF) proteins.
Antibacterial activity. The increasing amount of experimental data available in the literature show that there are some powerful links among the pathogen bacterial flora (i.e., septicemia) or the opportunistic agents (i.e., pneumonia infections) and certain cancer kinds like urogenital, cervical, stomach cancers, liver, and lymphoproliferative disturbances. Therefore, the pathogen bacterial flora and the opportunistic agents may be taken into consideration both in the management of cancer patients and in individual susceptibility to cancer. Indeed, dual acting factors with antimicrobial and antiproliferative potentials can result in improved therapeutic efficacy for cancer cell patients or reduced cancer predisposition. In light of this information, the antimicrobial activities of 1–4 were also tested against four gram-positive bacteria and five gram-negative bacteria. The experiments were conducted in triplicate to prevent possible errors, and SCF [Sulbactam (30 µg) + Cefoperazone (75 µg)] was used as a standard drug.

Results were $4 > 2 > 3 > SCF > 1 > KCN$ for antibacterial activities while for $S.\ enteridis$, and $S.\ gallinarum$ were $4 > SCF > 1 > KCN$ sequence of antibacterial effects (Tables 4 and 5). The bacterial inhibition sites of complexes

Figure 9. UV absorption spectra of 25 µM 1, 2, 3 and 4 in the absence (a) and presence of 6.25 µM (b), 12.5 µM (c), 25 µM (d), 50 µM (e), 100 µM (f), 200 µM (g), 800 µM (i) BSA.
1–4 are shown in Figures S20, S21 and S22 (Figures S20, S21 and S22 Table S3; Supplementary Material). The values of 36 and 37 mm made it obvious that the antibacterial effect of type of 4 was the strongest among all.

In this study, molecules 4 and 1 were subjected to MIC, and the findings profiles are submitted in Table 5. Sulbactam (30 µg) + Cefoperazone (75 µg) (105 µg/disc), were used as the standard and investigated by the Serial microdilution method to obtain MIC values in Mueller–Hinton Broth for the antibacterial test. The inhibition zones and MIC amounts for strains for 4 and 1 were recorded in the range of 15–37 mm and 15.62–125 µg/mL, respectively (Tables 5 and 4). Four types of gram-positive bacterial strains (St. pyogenez, B. subtilis, B. cereus, S. aureus) and five types of the gram-negative (S. enteridis, E. aerogenes, P. aeruginosa, E. coli, and S. gallinarum) were sensitive to 4 and 1. For the 4, the MIC and inhibition zones values of the bacterial strains were found as 31.25–125 µg/mL and 25–37 mm, respectively. In Table 5, molecule 1 (MIC: 15.62, 15.62, 31.25 µg/mL, respectively) exhibited better activities than molecule 4 and the standard for St. pyogenez, E. aerogenes, and S. gallinarum bacteria.

**Conclusion**

In this present study, four different complexes were synthesized using Ni²⁺ (1), Cu²⁺ (2), Zn²⁺ (3), Cd²⁺ (4), K [Ag(CN)₂]₁, and N-bishydeten and characterized by some advanced analytical techniques. Complex 4 consisting of [Cd(N-bishydeten)₄][Ag(CN)₂]₈[Ag(CN)] has a sandwich-type layered structure verified by the crystal method. In addition, the complexes were studied for their pharmacological properties, and they exhibited very strong anticancer (2–4) and antimicrobial activities (1–4). The compounds, especially 2, possessed more selective cytotoxic activity than the positive control against cancer cells, particularly HT29. The interaction of 1–4 with CT-DNA and BSA was shown with respect to the spectral changes in their absorbance, and their binding affinity was found to be very similar to the currently used anticancer agents such as cisplatin and 5FU. In future studies, we will try to improve the amount and functionality of our Ag(I) complexes using different ligands, metal salts, and new methods. Since the in vitro biological properties of these Ag(I) complexes can be used mainly against some cancer cell lines, in vivo anticancer study is very important to reveal the mechanism of action. In summary, our results show that these molecules are potentially valuable drug candidates and are suitable for further pharmacological testing.

**Table 4.** Antibacterial activity of 1–4 (105 µg/disc). SCF, sulbactam (30 µg) + cefoperazone (75 µg), as a positive control. KCN, potassium cyanide, as a negative control.

| Microorganisms                      | SCF | KCN | 1   | 2   | 3   | 4   |
|-------------------------------------|-----|-----|-----|-----|-----|-----|
| Gram-positive bacteria              |     |     |     |     |     |     |
| S. aureus ATCC29213                  | 29  | -   | 18 ± 0.58 | 37 ± 1.0 | 25 ± 0.58 | 30 ± 1.0 |
| B. subtilis ATCC6633                 | 19  | -   | 14 ± 1.53 | 27 ± 0.58 | 31 ± 1.0 | 34 ± 0.58 |
| B. cereus DSM 4312                  | 30  | -   | 20 ± 1.0  | 30 ± 0.58 | 34 ± 0.0  | 36 ± 1.0 |
| St. pyogenez ATCC1576               | 20  | -   | 15 ± 0.0  | 32 ± 0.58 | 29 ± 0.58 | 26 ± 0.0  |
| Gram-negative bacteria              |     |     |     |     |     |     |
| E. coli 111                         | 21  | -   | 19 ± 0.58 | 32 ± 0.0  | 30 ± 0.58 | 29 ± 0.58 |
| E. aerogenes 2924                   | 31  | -   | 15 ± 1.0  | 22 ± 1.0  | 20 ± 1.0  | 25 ± 1.0  |
| S. gallinarum ATCC9027              | 30  | -   | 17 ± 0.58 | 35 ± 1.0  | 35 ± 0.58 | 37 ± 1.0  |
| P. aeruginosa ATCC9027              | 15  | -   | 30 ± 1.0  | 30 ± 1.0  | 30 ± 0.58 |
| S. enteridis ATCC13076              | 22  | -   | 18 ± 0.58 | 30 ± 0.58 | 33 ± 0.58 | 36 ± 1.0  |

**Table 5.** Minimum-inhibitory concentrations (MIC, in mg/mL) of 1 and 4. SCF, sulbactam (30 µg) + cefoperazone (75 µg), as a positive control. KCN, potassium cyanide, as a negative control.

| Microorganisms                      | KCN | 1     | 4     | SCF  |
|-------------------------------------|-----|-------|-------|------|
| Gram-positive bacteria              |     |       |       |      |
| S. aureus ATCC29213                  | -   | 62.50 ± 36.08 | 62.50 ± 0.0 | 250  |
| B. subtilis ATCC6633                 | -   | 62.50 ± 0.0  | 31.25 ± 0.0 | 500  |
| B. cereus DSM 4312                  | -   | 62.50 ± 18.04 | 62.50 ± 18.04 | 500  |
| St. pyogenez ATCC1576               | -   | 15.62 ± 9.02 | 31.25 ± 9.02 | 500  |
| Gram-negative bacteria              |     |       |       |      |
| E. coli 111                         | -   | 62.50 ± 0.0  | 31.25 ± 0.0 | 250  |
| P. aeruginosa ATCC9027              | -   | 62.50 ± 18.04 | 62.50 ± 0.0 | 1000 |
| E. aerogenes ATCC2924               | -   | 15.62 ± 0.0  | 125 ± 53.40 | 62.50 |
| S. gallinarum ATCC9027              | -   | 31.25 ± 0.0  | 125 ± 0.0  | 62.50 |
| S. enteridis ATCC13076              | -   | 62.50 ± 17.97 | 62.50 ± 0.14 | 1000 |
Data Availability
X-ray graphic files in CIF format for 4 and crystallographic results for the chemical structure reported here have been deposited with the Cambridge Crystallographic Data Centre as supplementary data, CCDC Nos. 1519618. Copies of the data can be obtained through application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK. (Fax: + 44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk or at https://www.ccdc.cam.ac.uk).

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Author contributions
A.K., N.K., A.A., Ş.T., Y.Y., and F.Ş participated in the study design and coordination, conducted molecular studies and prepared the manuscript. All authors have read and approved the last article.

Competing interests
The authors declare no competing interests.

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