[Ag(L)NO₃] Complexes with 2-Benzoylpyridine-Derived Hydrazones: Cytotoxic Activity and Interaction with Biomolecules

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ABSTRACT: Complexes [Ag(H₂BzPh)NO₃] (1), [Ag(H₂BzCH₃Ph)NO₃] (2), [Ag(H₂BzCIPh)NO₃] (3), and [Ag(H₂BzNO₂Ph)NO₃] (4) were synthesized with 2-benzoylpyridine benzoylhydrazone (H₂BzPh) and its para-methyl-benzoylhydrazone (H₂BzCH₃Ph), para-chloro-benzoylhydrazone (H₂BzCIPh), and para-nitro-benzoylhydrazone (H₂BzNO₂Ph) derivatives. Experimental data indicate that the nitrate ligand binds more strongly to the silver center through one of the oxygen atoms, whereas the second oxygen atom from nitrate and the hydrazone oxygen makes much weaker interactions with the metal. Dissociation of nitrate most probably occurs in solution and in biological media. Interestingly, theoretical calculations suggested that when dissociation of the nitrate takes place, all bond orders involving the metal and the atoms from the hydrazone ligand increase significantly, showing that the bonding of nitrate results in the weakening of all other interactions in the metal coordination sphere. Upon complexation of the hydrazones to silver(I), cytotoxicity against B16F10 metastatic murine melanoma cells increased in all cases. Complexes (1–3) proved to be more cytotoxic than cisplatin. All compounds were more cytotoxic to B16F10 cells than to nontumorigenic murine Melan-A melanocyte cells. Interestingly, the selectivity index (SI = IC₅₀ non-malignant cells/IC₅₀ tumor cells) of complex (1), SI = 23, was much higher than that of the parent hydrazone ligand, SI = 9.5. Studies on the interactions of complexes (1–3) with DNA suggested that although (1–3) interact with calf thymus DNA by an intercalative mode, direct covalent binding of silver(I) to DNA probably does not occur. Complexes (1–3) interact in vitro with human serum albumin indicating that these compounds could be transported by albumin.

INTRODUCTION

Hydrazones and their metal complexes exhibit numerous pharmacological applications as antifungal, antibacterial, anti-inflammatory, and cytotoxic agents. 2-Benzoylpyridine-derived hydrazones present cytotoxic activity against several tumor cell lines.¹⁻³ In previous works, we demonstrated that upon coordination of these hydrazones to bismuth(III)⁴ and gallium(III),⁴ the cytotoxic effects against leukemia and solid tumor cells were improved in comparison to the free ligands.

Silver compounds are well-known for their pharmacological applications as antiseptic, antimicrobial, and an anti-inflammatory agents.⁵ Silver sulfadiazine is used worldwide in the treatment of skin infections in burns and wounds,⁶ although some disadvantages of silver sulfadiazine such as its cytotoxic effect on epidermal cells have been reported in recent years.⁷ Silver(I) complexes with different ligands proved to present antimicrobial properties.⁸⁻¹³ In addition, silver(I) complexes have become attractive as anticancer therapeutic drug candidates because of their antiproliferative activity and selectivity against numerous cancer cells.⁹¹²¹³

Considering the high cytotoxic activity of 2-benzoylpyridine-derived hydrazones previously investigated by us against neoplastic cells, in the present work, silver(I) complexes were synthesized with 2-benzoylpyridine benzoylhydrazone (H₂BzPh) as well as with its para-methyl-benzoylhydrazone (H₂BzCH₃Ph), para-chloro-benzoylhydrazone (H₂BzCIPh), and para-nitro-benzoylhydrazone (H₂BzNO₂Ph) analogues (Figure 1). The cytotoxic effects of the complexes under study were evaluated on B16F10 metastatic melanoma and against

Figure 1. Structural representation of 2-benzoylpyridine-benzoylhydrazones (E and Z isomers).

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RESULTS AND DISCUSSION

Formation of the Silver(I) Complexes. Microanalyses are compatible with the formation of [Ag(H2BzPh)NO3] (1), [Ag(H2BzpCH2Ph)NO3] (2), [Ag(H2BzpClPh)NO3] (3) and [Ag(H2BzpNO2Ph)NO3] (4), in which a hydrazone ligand is attached to the silver(I) center together with a nitrate ion. The relatively high molar conductivities in dimethyl sulfoxide (DMSO) suggest the probable release of nitrate in solution. In fact, crystal structure determinations of (1–3) and theoretical studies (see Supporting Information) are in accordance with a loosely bound nitrate ligand in all complexes (1–4).

Spectroscopic Characterization. Infrared Spectra. The vibrations assigned to ν(N−H) at 3283–3275 cm⁻¹ in the infrared spectra of the hydrazones are observed at 3354–3216 cm⁻¹ in the spectra of complexes (1–4). The ν(C=N) vibration at 1610–1600 cm⁻¹ in the spectra of the hydrazones are noticed at 1610–1594 cm⁻¹ in those of complexes (1–4), indicating coordination of the azomethine nitrogen. The ν(C=O) absorption at 1686–1650 cm⁻¹ in the spectra of the hydrazones shifts to 1678–1660 cm⁻¹ in complexes (1–4). The in-plane deformation mode of the pyridine ring at 614–628 cm⁻¹ in the spectra of the free hydrazones shifts to 630–623 cm⁻¹ in complexes (1–4), suggesting complexation of the heteroaromatic nitrogen. In addition, the vibration attributed to the nitrate group ν(NO3) was observed at 1384 cm⁻¹ and the ν(Ag(I)−O) vibration mode was found at 544–516 cm⁻¹ in the spectra of complexes (1–4).14,15

NMR Spectra. The NMR spectra of the hydrazones and their silver(I) complexes (1–3) were recorded in DMSO-d6. Because complex (4) undergoes ligand release in DMSO-d6, its NMR spectra were not recorded. The 1H resonances were assigned based on chemical shifts, multiplicities, and by using two-dimensional (2D) homonuclear 1H−1H correlation spectroscopy. The carbon type (C, CH) was determined employing distortionless enhancement by polarization transfer (DEPT 135) experiments and the attributions were made by means of 2D heteronuclear multiple quantum coherence and heteronuclear multiple bond coherence experiments.

In the 1H NMR spectra of the hydrazones, all signals were duplicated, indicating the existence of the Z and E configurational isomers in DMSO-d6 solution. In the first, N3−H is hydrogen-bonded to the pyridine nitrogen (δ 14.75−14.72), whereas in the latter, N3−H is hydrogen-bonded to the solvent (δ 10.37−10.04).5,16 The signals of all carbons are also duplicated in the 13C NMR spectra of the hydrazones, according to the presence of the Z and E isomeric forms in solution.3

In the spectra of complexes (1–3), only one signal was observed for each hydrogen and each carbon, consistent with the presence of only one isomer in solution. The signal of N3−H at δ 11.40−11.14 is characteristic of the E configuration. The signals of C7 and C8 undergo substantial shifts on coordination, in accordance with complexation through the imine nitrogen and the carbonyl oxygen. The signals of the pyridine carbons also undergo significant shifts upon complexation, indicating coordination through the heteroaromatic nitrogen. Interestingly, theoretical studies (see Supporting Information) suggested a strengthening of the silver(I)−oxygen (from the hydrazone) bond upon release of the nitrate ligand in solution, which is compatible with the observed shifting of the C8=O signal in the spectra of the complexes in comparison to those of the free hydrazone ligands.

X-ray Diffraction Analyses. Molecular plots for (1–3) are shown in Figure 2. Table 1 shows selected bond distances and angles in the structures of (1–3). Crystal data and refinement results are in Table S1, Supporting Information.

In complexes (1–3), the distances and angles in the hydrazone skeleton are similar. However, comparison of the bond distances in H2BzpClPh4 and in complex (3) reveals substantial changes due to complexation.

In (1–3), a hydrazone ligand is attached to the silver(I) center together with a nitrate ion. In all complexes, the Ag1−O1 [2.6048(15) (1), 2.7110(19) (2), and 2.632(2) (3)] and Ag1−O3 [2.6331(19) (1), 2.774(2) (2), and 2.739(3) (3)] distances are longer than the Ag−O2 bond lengths [2.3565(19) (1), 2.2789(19) (2), and 2.286(3) (3)]. Therefore, weaker interactions were found between the metal center and the hydrazone carbonyl oxygen and between the metal center and O3 from the nitrate than between the silver(I) center and O2 from the nitrate. This effect is consistent with the high molar conductivities of complexes (1–3), which might be due to the release of the loosely bound nitrate ligand in solution.

Computational Studies. The results and discussion of the theoretical studies on the structural properties of complexes (1–4) and on the nature of the Ag−L bonds are in the Supporting Information.

Cytotoxic Activity. The cytotoxic effects of the hydrazones and their silver(I) complexes (1–3) were evaluated in comparison with cisplatin on B16F10 (metastatic melanoma) and Melan-A (nontumorigenic melanocyte) cells. All compounds revealed to be more cytotoxic than cisplatin and silver nitrate. All compounds revealed to be more cytotoxic to B16F10 cells than to the nontumorigenic Melan-A cells. Interestingly, the selectivity index (SI = IC50 non-malignant cells/IC50 tumor cells) of complex (1), SI = 23, was much higher than that of the parent hydrazone ligand, SI = 9.5.
Table 1. Selected Bond Distances (Å) and Angles (°) for [Ag(H2BzPh)NO3] (1), [Ag(H2BzpCH3Ph)NO3] (2), and [Ag(H2BzpC1Ph)NO3] (3) along with Data for H2BzpC1Ph

| Bond Distances | (1)          | (2)          | H2BzpC1Ph   |
|----------------|--------------|--------------|-------------|
| N1–C2          | 1.336(3)     | 1.352(3)     | 1.353(3)    |
| C2–C7          | 1.488(3)     | 1.481(4)     | 1.484(2)    |
| C7–N2          | 1.290(3)     | 1.297(3)     | 1.302(2)    |
| N2–N3          | 1.368(2)     | 1.362(3)     | 1.369(19)   |
| N3–C8          | 1.367(3)     | 1.380(3)     | 1.366(2)    |
| C8–O1          | 1.224(2)     | 1.220(3)     | 1.215(2)    |
| Ag1–N1         | 2.3807(18)   | 2.366(2)     | 2.439(3)    |
| Ag1–N2         | 2.3957(17)   | 2.388(2)     | 2.316(3)    |
| Ag1–O1"       | 2.6048(15)   | 2.7110(19)   | 2.632(2)    |
| Ag1–O2         | 2.3565(19)   | 2.2789(19)   | 2.286(3)    |
| Ag1–O3"       | 2.6331(19)   | 2.774(2)     | 2.739(3)    |

Table 2. IC50 and SI Values for the Hydrazones and Complexes (1–3) against B16F10 Cells

| compound                      | IC50 ± SD (μM) and SI | B16F10 | Melan-A | SI |
|-------------------------------|-----------------------|--------|---------|----|
| H2BzPh                        | 11.50 ± 0.01          | 109.30 ± 0.01 | 9.5  |
| [Ag(H2BzPh)NO3]               | 2.36 ± 0.01           | 54.63 ± 0.01 | 23   |
| H2BzpCH3Ph                    | 4.00 ± 0.02           | 96.30 ± 0.01 | 24   |
| [Ag(H2BzpCH3Ph)NO3]           | 2.00 ± 0.01           | 10.23 ± 0.04 | 5.1  |
| H2BzpC1Ph                     | 5.00 ± 0.01           | >100.00    |       |
| [Ag(H2BzpC1Ph)NO3]            | 2.00 ± 0.04           | 7.87 ± 0.04 | 3.9  |
| AgNO3                         | 75.40 ± 0.01          | 59.90 ± 0.01 | 0.8  |
| cisplatin                     | 10.00 ± 0.01          | >100.00    |       |

“IC50 = concentration that reduced 50% of cell proliferation. SD = standard deviation. SI = IC50 non-malignant cell/IC50 tumor cell.

DNA Binding Studies. The electronic spectra of complexes (1–3) were registered in the absence and in the presence of increasing concentrations of calf thymus DNA (CT-DNA). Upon addition of DNA, a slight hypochromism and a small bathochromic shift were noticed at the wavelength of maximum absorption (Figure 3a), indicating intercalative binding. Hypochromism probably occurs due to π-π stacking interactions between aromatic groups and the DNA base pairs, as in the case of classical intercalators such as ethidium bromide (EB).17

With the purpose to quantitatively compare the binding strength, the intrinsic binding constants (Kb) of (1–3) with CT-DNA were determined as previously described.18 As shown in Figure 3b, the plot of [DNA]/(εb − εa) versus [DNA] gives 1/(εb − εa) as the slope and 1/(Kb(εb − εa)) as the intercept. [DNA] is the concentration of DNA base pairs, εa is the molar absorption coefficient of the complex at a given DNA concentration, and εb and εa are the molar absorption coefficients of the complex unbound and completely bound to DNA, respectively.

The intrinsic binding constant Kb is determined as the ratio between the slope and the intercept. The calculated Kb values are shown in Table 3. All complexes presented Kb values on the order of 103 M−1.

Competitive binding experiments were performed to obtain further information on the interactions of complexes (1–3) with DNA. Figure 4a represents the emission spectra of EB bound to DNA in the absence and presence of complex (1). Upon excitation at 545 nm, the EB–DNA system exhibits a strong emission at 602 nm. A decrease in the emission intensity was found in the presence of complexes (1–3), probably due to the
Table 3. Intrinsic Binding Constant (K_{in}) Concentration Required To Reduce 50% of the Fluorescence of EB–DNA System (C_{50}), and Apparent Binding Constant (K_{app}) for the Competitive Binding between EB Bound To CT-DNA and Silver(I) Complexes (1–3)

| compound                  | K_{in} (10^6 M^{-1}) | C_{50} (10^{-3} M) | K_{app} (10^3 M^{-1}) |
|---------------------------|----------------------|--------------------|-----------------------|
| [Ag(H2BzPh)NO3]           | (1)                  | 2.6 ± 0.4          | 21.0                  |
| [Ag(H2BzCPH)NO3]          | (2)                  | 2.3 ± 0.3          | 19.8                  |
| [Ag(H2BzPhClPh)NO3]       | (3)                  | 2.2 ± 0.7          | 17.9                  |

Figure 4. (a) Fluorescence quenching spectra (λ_{ex} = 545 nm) for EB–DNA in the absence (black line) and presence (color lines) of increasing amounts of [Ag(H2BzPh)NO3] (1). The arrows show the changes in intensity at increasing concentrations of (1). (b) Stern–Volmer plots of relative EB–DNA fluorescence intensity F_0/F vs [complex] (1–3). ([complexes] = 0–100 μM, [DNA] = 10 μM, and [EB] = 10 μM).

Figure 6. (a) Fluorescence spectra of HSA (1.84 μM, λ_{ex} = 295 nm) with increasing concentrations of [Ag(H2BzPh)NO3] (1) (0–6 μM) at 298 K. The arrows indicate the spectral changes. (b) Stern–Volmer plots of F_0/F vs [complex] for the binding between complex (1) and HSA at different temperatures. (c) Plots of log(F_0/F) vs [complex] for the determination of the binding constants (K_{app}) and number of active sites (n) at different temperatures. (d) van’t Hoff plots of ln K vs 1/T for the binding silver(I) complexes (1–3) with HSA.

Interactions on site I can be monitored by investigating the fluorescent properties of the tryptophan residue (Trp-214) located in the IIA subdomain. Fluorescence spectra using λ_{ex} ≥ 295 nm are due entirely to this residue.22

The emission spectra of HSA were recorded at 298 K in the absence and in the presence of several concentrations of complex (1) (Figure 6a). HSA shows strong emission at 342 nm (excitation at 295 nm) upon binding with increasing concentrations of (1), with a fluorescence maximum at 342 nm and a decrease in fluorescence intensity at increasing concentrations of (1). The Stern–Volmer quenching constant (K_{sv}) for the determination of the binding constants (K_{app}) and number of active sites (n) at different temperatures. (d) van’t Hoff plots of ln K vs 1/T for the binding silver(I) complexes (1–3) with HSA.

Figure 5. Agarose gel electrophoresis of pUC19 plasmid DNA from Escherichia coli (150 ng μL^{-1}) incubated with H2BzPh (L1), H2BzCPH,Ph (L2), H2BzCPH (L3), [Ag(H2BzPh)NO3] (1), [Ag(H2BzCPH,Ph)NO3] (2), [Ag(H2BzCPH)NO3] (3), AgNO3, and cisplatin (100 μM) for 24 h at 37 °C.

Reduction in the number of binding sites on DNA accessible to EB.

The spectral modifications suggest that the complexes interact with DNA, at least in part by an atypical intercalating process.19 The apparent binding constants (K_{app}) for complexes (1–3) were calculated as previously reported18 from the equation K_{app}[EB] = K_{app}[complex], where K_{app} is 1.0 × 10^6 M^{-1}, [EB] = 10 μM, and [complex] is the complex concentration when the fluorescence intensity of EB is 50% of the initial fluorescence. This value is obtained from the plot F_0/F versus [complex] when F_0/F = 2 (Figure 4b, Table 3). The determined K_{app} values are similar to that reported for the antineoplastic intercalating agent actinomycin D (K_{app} = 9.69 × 10^6 M^{-1}).20

The effects of the hydrazine ligands, complexes (1–3), AgNO3, and cisplatin on DNA conformation were evaluated by the analyses of the electrophoretic mobility of pUC19 plasmid DNA after interaction with the compounds (Figure 5). At the employed concentration (100 μM), the hydrazine ligands, AgNO3, and complexes (1–3) did not interact with DNA. At the same concentration, cisplatin significantly interacted with DNA, hindering its electrophoretic mobility.

Albumin Binding Studies. The literature reports that most chemotherapeutic compounds bind to site I (located on IIA subdomain) and site II (located on IIIA subdomain) on HSA.21
of the quencher \((\tau _{0} = 10^{-8} \, \text{s})\),\(^{26}\) and \([Q]\) is the concentration of the quencher \([Q] = \text{[complex]}\). According to eq 2:

\[
K_{sv} = k_{q0}
\]

(2)

\(K_{sv}\) is determined as the slope of the plot of \(F_0/F\) versus \([\text{complex}]\) (Figure 6b). The binding constant \((K_b)\) and the number of binding sites \((n)\) are calculated by means of eq 3:

\[
\log ((F_0 - F)/F) = \log K_b + n \log [Q]
\]

(3)

where \(K_b\) is the binding constant of the quencher with HSA, \(n\) is the number of binding sites, and \(F_0\) and \(F\) are the fluorescence intensity in the absence and the presence of the quencher, \(Q\). The plot of \(\log ((F_0 - F)/F)\) versus \(\log [\text{complex}]\) gives \(n\) and \(K_b\) as the slope and the intercept, respectively (Figure 6c).

The calculated Stern–Volmer quenching constants (Table 4) correlate inversely with temperature. \(K_b\) values on the order of \(10^{12} \, \text{M}^{-1} \, \text{s}^{-1}\) were higher than typical diffusion-controlled quenching rates of molecules in aqueous medium, \(2.0 \times 10^{10} \, \text{M}^{-1} \, \text{s}^{-1}\).\(^{24}\) Therefore, the fluorescence quenching did not result from dynamic collisions but might have been provoked by a specific interaction between HSA and complexes (1–3), consistent with a static quenching mechanism. The binding constants decreased with increasing temperature, resulting from a decrease in the stability of the HSA–Ag(I) system. The \(n\) values close to 1 indicate that the complexes bind HSA in 1:1 molar ratio.

The nature of the forces that direct the interactions with HSA can be inferred from the analysis of the thermodynamic parameters of the process.\(^{27}\) Figure 6d shows the van’t Hoff diagram \((\ln K \text{ vs } 1/T)\) for the interaction between HSA and complexes (1–3). The standard enthalpy change \((\Delta H^\circ)\) and the standard entropy change \((\Delta S^\circ)\) were obtained from the van’t Hoff equation by plotting \(\ln K_b\) versus \(1/T\), where \(-\Delta H^\circ/R\) is the angular coefficient and \(\Delta S^\circ/R\) is the linear coefficient.\(^{28}\) The negative variations in standard enthalpy \((\Delta H^\circ)\) and standard entropy \((\Delta S^\circ)\) suggest the occurrence of van der Waals forces and/or hydrogen bonds. The negative values for \(\Delta G\) indicate a spontaneous binding process.

### CONCLUSIONS

In complexes \([^\text{Ag(H2BzPh)NO}_3\] (1), \([^\text{Ag(H2BzpCH}_3\text{Ph})\text{NO}_3\] (2), \([^\text{Ag(H2BzpClPh)NO}_3\] (3), and \([^\text{Ag(H2BzpNO}_3\text{Ph})\text{NO}_3\] (4), one hydrazone (L) and one nitrate ligand are attached to the silver(I) center. Experimental and theoretical (reported in Supporting Information) data suggested that although one of the nitrate oxygens binds more strongly, the second oxygen from nitrate and the hydrazine oxygen make much weaker interactions with the metal. Similarly, experimental and theoretical data revealed that nitrate dissociation probably occurs in solution and in biological milieu. In addition, nitrate dissociation results in the strengthening of all Ag–L bonds.

Although the cytotoxic effects of silver(I) complexes against tumor cells have not been fully investigated, the recent literature reports that these compounds exhibit antiproliferative activity against several tumor cell lineages. In some cases, the compounds have been shown to be more cytotoxic to malignant than to nonmalignant cells.\(^{5}\)

Complexes (1–3) proved to have higher cytotoxic activity against B16F10 (metastatic melanoma) cells than the free hydrazine ligands and revealed to be more cytotoxic than cisplatin. All compounds under study were more cytotoxic to B16F10 cells than to the nonmalignant Melan-A cells. Interestingly, the SI of complex (1), SI = 23, was much higher than that of the parent hydrazine ligand, SI = 9.5, suggesting that in this case, coordination to silver(I) was a good strategy for improving selectivity.

Complexes (1–3) interact with DNA probably by an intercalative mode. This interaction might be favored by the presence of the benzene or pyridine aromatic rings in the ligand skeleton. In spite of the possible ability of complexes (1–3) to interact with DNA by an intercalative process, the silver(I) center probably does not covalently bind to DNA. Hence, covalent binding of silver(I) to DNA is probably not part of the mechanism of cytotoxic action of these compounds.

The literature reports that silver(I), when assayed as silver nitrate aqueous solution, covalently binds to N7 of guanine and adenine.\(^{29}\) The foregoing results suggest that, when bound to the hydrazones, silver(I) does not show high ability to bind to the DNA bases. Hence, unlike cisplatin, the complexes under study probably do not favor direct coordination of silver(I) to DNA.

In addition, the present investigation revealed that complexes (1–3) interact with HSA (free-energy values on the order of \(-20 \, \text{kJ mol}^{-1}\)), indicating that these compounds could be transported by albumin in the blood.

Considering that the pharmacological profile of silver(I) complexes remains poorly investigated, the present study constitutes an important contribution to the understanding of the possible applications of silver(I)-based compounds as anticancer drug candidates.

Moreover, the theoretical investigations (see Supporting Information) provided new insights into the nature of silver(I) coordination chemistry.
EXPERIMENTAL SECTION

Materials and Measurements. All common chemicals were purchased from Aldrich and were used as received. Microanalyses were performed on a PerkinElmer CHN 2400 analyzer. Melting points were determined with a Mettler MQAPF-302 apparatus. Molar conductivities \( (\lambda_m) \) were obtained with a Bruker_DPX-400 ADVANCE (400 MHz) spectrometer employing KBr pellets (4000−1600 cm\(^{-1}\)) and the structure of (C11), (C13), 129.0 ([C11], [C13]), 21.0 (C21). Yield: 68%.

Electronic spectra were recorded using a Shimadzu UV−2401PC UV−vis spectrophotometer employing 1 cm quartz cells. Fluorescence spectra were recorded on a Varian Cary Eclipse spectrophotometer using a 1 cm quartz cell. NMR spectra were obtained with a Bruker DPX-400 ADVANCE (400 MHz) spectrometer using DMSO−d\(_6\) as the solvent and tetramethylsilane as the internal reference. Single-crystal X-ray diffraction measurements were carried out on an Oxford-Diffraction GEMINI-Ultra diffractometer (LabCry-UMFG) using graphite-Enhance Source Mo K\(\alpha \) radiation \((\lambda = 0.71073 \ \text{Å})\). Data were collected at 100 (1), 120 (2), and 270 K (3). Data collection, cell refinements, and data reduction were performed using the CrysalisPro software. The CRYSTALS suite was used for analytical absorption corrections. The structures of (1) and (3) were solved using SUPERFLIP and the structure of (2) was solved using SIR92. Full-matrix least-squares refinement procedure on \( F^2 \) with anisotropic thermal parameters was carried on using SHELXL-2017. Positional and anisotropic atomic displacement parameters were refined for all non-hydrogen atoms. Hydrogen atoms were placed geometrically and the positional parameters were refined using a riding model.

Syntheses of the Hydrazones and Their Silver(I) Complexes. The hydrazones were prepared as previously described. The silver(I) complexes (1−4) were synthesized by mixing a methanol solution (10 mL) of the desired hydrazone (1.0 mM) with an aqueous solution of AgNO\(_3\) (3 mL) added dropwise in equimolar amount. The reaction mixture was maintained in the dark at room temperature for 72 h. The obtained solids were filtered off, washed with methanol and diethyl ether, and then dried under reduced pressure.

[2-Benzoylpyridine-benzoylhydrazone-nitrile]silver(I) \([\text{Ag}(\text{H2BzPh})\text{NO}_3])\ (1). Gray solid. Anal. Calcld for \( \text{C}_{19}\text{H}_{14}\text{N}_5\text{O}_6\text{Ag} \): C, 44.21; H, 2.73; N, 13.57. Found (%): C, 45.20; H, 2.85; N, 13.66. FW: 516.21 g mol\(^{-1}\). Molar conductivity (1.0 mM, DMSO): 48.7 \( \Omega^{-1}\) cm\(^{-1}\) mol\(^{-1}\).

[2-Benzoylpyridine-benzoylhydrazone-nitro]silver(I) \([\text{Ag}(\text{H2BzNO}_2\text{Ph})\text{NO}_3])\ (2). Gray solid. Anal. Calcld for \( \text{C}_{19}\text{H}_{14}\text{N}_5\text{O}_6\text{Ag} \): C, 45.13; H, 2.79; N, 11.08. Found (%): C, 45.20; H, 2.85; N, 11.10. FW: 505.66 g mol\(^{-1}\). Molar conductivity (1.0 mM, DMSO): \( 37.6 \Omega^{-1}\) cm\(^{-1}\) mol\(^{-1}\).

Crystal Structure Determination. Crystals of (1−3) were obtained from the mother liquor in the syntheses of (1−3) at room temperature. The crystal structures were determined using single-crystal X-ray diffractometry. A summary of the crystal data, data collection details, and refinement results for these compounds is listed in Table S1, Supporting Information. Molecular graphics were prepared using ORTEP 3 for Windows.

THEORETICAL CALCULATIONS

Computational Details. Computational Details are in the Supporting Information.

Cytotoxic Activity. Cell Cultures. B16F10 metastatic murine melanoma cells were received as a gift from Ludwig Institute for Research about Cancer (São Paulo, Brazil) and the nonmalignant murine melanocyte cell line (Melan-A) was provided by Dr. Roger Chamas, Cancer Institute (São Paulo, Brazil). Cells were cultured in RPMI 1640 medium (Cultilab) supplemented with 10% fetal bovine serum (FBS) (Gibco) and 1% penicillin/streptomycin (Sigma). B16F10 cells were received as a gift from Ludwig Institute for Research about Cancer (São Paulo, Brazil) and the nonmalignant murine melanocyte cell line (Melan-A) was provided by Dr. Roger Chamas, Cancer Institute (São Paulo, Brazil). Cells were cultured in RPMI 1640 medium (Cultilab) supplemented with 10% fetal bovine serum (FBS) (Gibco) and 1% penicillin/streptomycin (Sigma). B16F10 cells were cultured at pH 7.4 and Melan-A cell culture was prepared at pH 6.9 in the presence of 200 nM Phorbol 12-myristate 13-acetate (Sigma). The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO\(_2\).

Evaluation of the Cytotoxic Activity. Cells were seeded in 96-well plates (B16F10−1 × 10\(^4\) cells/well and Melan-A−6 × 10\(^4\) cells/well). After complete adhesion of the cells on surface plate, the medium was replaced with fresh medium containing the test compounds in concentrations ranging from 100 to 0.1 \( \mu \)M. After 72 h of incubation, 10 \( \mu \)L of MTT (Sigma−5 mg/mL) was added in each well and the cells were kept for 4 h at
culture conditions. Cell viability was evaluated by determining the rate of mitochondrial reduction of MTT to formazan. The formazan precipitate was dissolved after replacing the culture medium by DMSO (100 μL/well). Subsequently, the absorbance at 570 nm was measured on an ELISA reader (Thermo Plate Reader-TP).

Control tests were performed by incubating the cells with 1% (v/v) of DMSO (negative control) in the maximum concentration employed in the experiment, and it was observed that the cellular viability was not affected by the solvent under these conditions. Cisplatin was used as the positive control.

The results were expressed as percentage of viable cells. The control containing RPMI 1640 and 10% FBS was considered to result in 100% cell viability. The IC50 values (μM) were calculated by nonlinear regression using the GraphPad Prism software. Statistical analysis was carried out by means of the one-way analysis of variance test, followed by Student-Newman-Keuls post-test. The acceptable level of significance was 95% (p < 0.05).

Interactions of Complexes (1–3) with CT-DNA.

Electronic Absorption Spectral Studies. The interactions of complexes (1–3) with CT-DNA were investigated employing electronic spectroscopy. CT-DNA was dissolved in Tris-HCl buffer (NaCl 50 mM/Tris-HCl 5 mM, pH 7.2) by shaking in an orbital shaker at 120 rpm at 37 °C for 24 h. The concentration of CT-DNA was calculated at 260 nm by using the molar extinction coefficient (ε) of 6600 M−1 cm−1.38 The stock solutions of the compounds were prepared in DMSO at 1.0 mM and subsequently diluted in Tris-HCl buffer. Titration experiments were registered in the 240–400 nm range by keeping the concentration of the complexes constant (30 μM), while progressively increasing the concentration of CT-DNA (0–20 μM) at ambient temperature. After each addition, the solutions were allowed to stand in equilibrium for 5 min before recording the spectra.

Competitive Binding between Complexes (1–3) and EB for CT-DNA. Competitive binding between EB and complexes (1–3) for CT-DNA was studied by fluorescence spectroscopy at ambient temperature. DNA–EB working solution (3.0 mL) (10 μM of CT-DNA with 10 μM of EB, prepared in Tris-HCl buffer) was titrated by consecutive addition of the complexes (1.0 mM, stock solution freshly prepared in DMSO). After each addition, the solution was stirred and allowed to stand for 5 min at the experimental temperature (298, 303, and 310 K). The emission spectra were registered in the 300–550 nm range with excitation at 295 nm.42

The excitation and emission slit widths and scan rates were maintained in all experiments. The amount of DMSO was maintained less than 3% (by volume) and had no effect on any experimental result. The fluorescence spectra (Figure 6a) were recorded using solutions of the compounds at low concentration, where the absorbances of HSA and of complexes (1–3) at 342 nm are <0.01%. Hence, the inner filter effect could be disregarded and fluorescence intensities were not corrected.43

**ASSOCIATED CONTENT**

 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b00533.

Fourier-transform infrared spectra of the hydrzones and their silver(1) [Ag(H2BzPh)NO3] (1), [Ag(H2BzPhCH2Ph)NO3] (2), [Ag(H2BzPPhCN)NO3] (3) and [Ag(H2BzPPh2)NO3] (4) complexes, as well as the 1H and 13C NMR spectra of 1–3 and X-ray crystallography data for complexes (1–3), computational results, and discussion for complexes (1–4), with detailed quantum theory of atoms in molecule analysis performed for complex (2) (PDF)

Supplementary crystallographic data for complex 1 (CIF)
Supplementary crystallographic data for complex 2 (CIF)
Supplementary crystallographic data for complex 3 (CIF)

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**Notes**

The authors declare no competing financial interest.

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