Triethylphosphinegold(I) Complexes with Secnidazole-Derived Thiosemicarbazones: Cytotoxic Activity against HCT-116 Colorectal Cancer Cells under Hypoxia Conditions

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ABSTRACT: Triethylphosphinegold(I) complexes \([\text{Au(HL1)P(CH}_2\text{CH}_3)_3\text{]}\text{PF}_6\) (1), \([\text{Au(HL2)P(CH}_2\text{CH}_3)_3\text{]}\text{PF}_6\) (2), and \([\text{Au(HL3)P(CH}_2\text{CH}_3)_3\text{]}\text{PF}_6\) (3) were obtained with \((E)-2-(1-(2\text{-methyl-5-nitro-1H-imidazol-1-yl})\text{propan-2-ylidene})\text{hydrazinecarbothioamide}\) (HL1), \((E)-\text{N-methyl-2-(1-(2\text{-methyl-5-nitro-1H-imidazol-1-yl})\text{propan-2-ylidene})hydrazinecarbothioamide}\) (HL2), and \((E)-2-(1-(2\text{-methyl-5-nitro-1H-imidazol-1-yl})\text{propan-2-ylidene})\text{-N-phenylhydrazinecarbothioamide}\) (HL3). All compounds were assayed for their cytotoxic activities against HCT-116 colorectal carcinoma cells under normoxia and hypoxia conditions and against nonmalignant HEK-293 human embryonic kidney cells under normoxia conditions. The thiosemicarbazone ligands HL1-HL3 were inactive against HCT-116 cells under hypoxia while but HL3 was inactive, HL1 and HL2 proved to be cytotoxic to both cell lineages under normoxia conditions. Complexes (1–3) and the triethylphosphinegold(I) precursor proved to be active against both cell lineages in normoxia as well as in hypoxia. While 1 and 3 revealed to be active against HEK-293 and HCT-116 cells, being approximately as active against HCT-116 cells in normoxia as under hypoxia, complex (2) proved to be more active against HCT-116 cells under hypoxia than under normoxia conditions, and more active against HCT-116 cells than against the nonmalignant HEK-293 cells, with the selectivity index, calculated as \(\text{SI} = \frac{\text{IC}_{50\text{HEK-293}}}{\text{IC}_{50\text{HCT-116hypoxia}}}\) equal to 3.7, similar to the value obtained for the control drug tirapazamine (tirapazamine (TPZ), SI = 4). Although the compounds showed distinct cytotoxic activities, the electrochemical behaviors of HL1-HL3 were very similar, as were the behaviors of complexes (1–3). Complex (2) deserves special interest since it was significantly more active under hypoxia than under normoxia conditions. Hence, in this case, selective reduction of the nitro group in a low oxygen pressure environment, resulting in toxic reactive oxygen species (ROS) and damage to DNA or other biomolecules, might operate, while for the remaining compounds, other modes of action probably occur.

INTRODUCTION

Thiosemicarbazones are an important class of bioactive compounds that show antimicrobial, antiviral, antiparasitic, and antineoplastic activities.1 Functionalization of thiosemicarbazones with a variety of pharmacophoric groups aiming to design new drug candidates has been extensively investigated.2,3 Nitroimidazole-containing compounds show antimicrobial,4,5 antiparasitic,6 and antineoplastic7 activities related to their ability to undergo a reduction of the nitro group, generating toxic reactive oxygen species (ROS) such as the nitro anion radical, \(\text{NO}_2^-\), under conditions of low oxygen concentration, which result in damage of DNA and other biomolecules. Hence, the presence of nitroimidazole may result in hypoxic selectivity.8,9 Hyoxia is frequently a characteristic of late-stage solid tumors. In solid tumor tissues, which grow and proliferate, oxygen consumption is higher than oxygen supply and transport, and the distance between cells and the abnormal vasculature increases, weakening oxygen circulation.9,10 Hence, hypoxia-selective compounds may in principle exhibit anticancer effects with reduced systemic toxicity.

Metal complexes with thiosemicarbazones also show pharmacological applications as antineoplastic11–13 antimicrobial,14 antiparasitic agents,15,16 and antiviral17 agents.

Gold(I, III) complexes with different ligands have shown in vitro and in vivo antitumor activities.18 Gold(I) and gold(III) complexes with thiosemicarbazones and bis(thiosemicarbazones) have been shown to exhibit antiprolifer-
ative activity against cancer cells and to act as inhibitors of the seleno-enzyme thioredoxin reductase (TrxR).

Auranofin, triethylphosphinegold(2,3,4,6-tetra-O-acetyl-β-1-d-thiopyranosato-S)gold(I), was the first metal–phosphine complex to be introduced into clinics for the treatment of rheumatoid arthritis. After the introduction of auranofin for the treatment of rheumatoid arthritis, numerous gold(I) complexes with thiolate, phosphine, N-heterocyclic carbene (NHC), and thiourea have been evaluated for their antiproliferative activities. In a previous work, we demonstrated that gold(I) complexes with 2-acetylpyridine- and 2-benzoylpyridine-derived thiosemicarbazones were cytotoxic to leukemia and solid tumor cells.

We also demonstrated that hydrazones and thiosemicarbazones derived from secnidazole, a 5-nitroimidazole-containing compound, as well as their copper(II), silver(I), and bismuth(III) complexes were inactive against aerobic bacteria but proved to be highly active against anaerobic strains, indicating that reduction of the nitro group under low oxygen concentrations might be part of their antimicrobial mode of action.

Tirapazamine (TPZ) is a hypoxia-activatable bio-reducible prodrug, which can be transformed from a nontoxic into a toxic species via a one-electron reduction in the hypoxic environment, resulting in selective antineoplastic effects against hypoxic tumors with minimal toxicity to nonmalignant tissues.

In the present work, triethylphosphinegold(1) complexes were obtained with secnidazole-derived thiosemicarbazones (Figure 1) and their cytotoxic activities against solid tumor and healthy cells under normoxia and hypoxia conditions were evaluated in comparison with TPZ.

**RESULTS AND DISCUSSION**

**Crystal Structure Determinations.** The crystal data analysis indicates that HL2 crystallized in the $P-1$ triclinic, while HL3 crystallized in the $Pbcn$ orthorhombic space group. HL2 formed twin crystals with the same composition and unit cell but with different orientations, indicating a pseudomerohedral twin. Of all reflections, 45.9% were indexed as component one, 20.4% as component two, 18% as overlapping reflections belonging to both components, and 15.7% were nonindexed.

Selected bond distances and angles in the structures of HL2 and HL3 are shown in Table 1. In both compounds, the C6-S and N3-C5 bond distances (1.689(4) and 1.275(5) Å for HL2 and 1.6709(16) and 1.272(2) Å for HL3, respectively) indicate the presence of C=Sn and C≡N double bonds, as expected. Structural representations and the atom numbering scheme for HL2 and HL3 are shown in Figure 2. The molecular conformation in both compounds is similar. The dihedral angle between the imidazole ring and the thiosemicarbazone fragment is 77.2(1)° in HL2 and 83.88(5)° in HL3. In HL3, the dihedral angle between the benzene ring and the thiosemicarbazone fragment is 14.43(8)°.

The crystal packings of the compounds are very distinct, mainly due to the presence of water molecules and of the phenyl ring in HL3 (see Figures S1 and S2, Supporting Information). In the crystal packing of HL2, an N5-H···N1 intermolecular hydrogen bond was observed (d(N5···N1) = 2.960(6)Å), with the formation of a dimeric motif (see Table S1, Supporting Information). An N4-H···S interaction is also observed in this compound (d(N4···S) = 3.457(4)Å) that gives rise to a one-dimensional (1-D) network arrangement. In the crystal packing of HL3, strong intermolecular interactions among HL3 molecules were not observed and stabilization of the solid is due to N4-H···O3 (d(N4···O3) = 2.937(2)Å and O3-H···N1 (d(O3···N1) = 2.853(2)Å) intermolecular hydrogen bonds among HL3 and water molecules (see Table S2, Supporting Information).

The Hirshfeld surfaces (HS) as the fingerprint plot analysis show that changing from methyl in HL2 to phenyl in HL3 promotes relevant modifications in the solid state. Comparing the HS of the compounds (Figure 3), it is possible to notice the difference in their shapes and, as
expected, in volume (HL2 = 314.64 Å³ and HL3 = 388.79 Å³). The distribution of the intermolecular interactions is also distinct in the compounds. In HL3, the intermolecular interactions are stronger than those in HL2, since the $d_i + d_e$ distance is 4.7 Å in HL2 and 4.5 Å in HL3. However, the strongest interactions are related to N···H contacts in both compounds. The most representative interactions in these solids are the nondirectional H···H contacts that represent 39.1 and 39.9% for HL2 and HL3, respectively (Figure S3a). The O···H contacts’ contributions (Figure S3b) are similar in HL2 and HL3: 20.5% in HL2 and 22.1% in HL3, respectively. However, due to the presence of water molecules, the fingerprint plots in HL3 are asymmetric for these contacts as well as for the N···H contacts (Figure S3c). The N···H and S···H contributions correspond to 14.2 and 15.3%, respectively, for HL2 and 5.0 and 9.0% for HL3. The fingerprint plots did not show the characteristic wing pattern, indicating that CH···π interactions are not present in these compounds.

**Formation of the Triethylphosphinegold(I) Complexes.** C, H, and N analyses, as well as molar conductivity data, are in accordance with the formation of [Au(HL)P-(CH2CH3)3]PF6 complexes in which one thiosemicarbazone ligand and one triethylphosphine ligand are attached to the gold(I) center.

**Spectroscopic Characterization. Infrared Spectra.** The vibrations attributed to $\nu$(C=S), observed at 804−772 cm$^{-1}$ in HL1−HL3, could not be attributed in 1−3 due to overlapping with the PF6 vibrations. However, the $\nu$(C=S−N) absorption, observed at 1530−1555 cm$^{-1}$ in the infrared spectra of the free bases, shifts to 1528−1540 cm$^{-1}$ in those of complexes (1−3), probably as a consequence of coordination through the sulfur.

Figure 2. Molecular plots of HL2 and HL3 showing the labeling scheme of the non-H atoms and their displacement ellipsoids at the 50% probability level.

Figure 3. Hirshfeld surface (top) and fingerprint plots (bottom) of (a) HL2 and (b) HL3.
observed at δ 147.0 δ 1137.

The EpIc values indicate that the formation of NO2/R− at 250 mV s−1.

This signal was found at δ 500 mV, assigned to the formation of a stable nitro radical anion (R−NO2). The voltammograms also exhibit a wave near −2000 mV, assigned to the generation of hydroxylamine (R−NOH) and, in some cases, an irreversible oxidation close to −500 mV, assigned to the formation of nitroso (R−NO) species (see eqs 1–3).

Peaks Ic and Ia R−NO2 + e− ⇄ R−NO2− (1)
Peak IIc R−NO2− + 3e− + 4H+ → R−NOH + H2O (2)
Peak IIIa R−NOH ⇄ R−NO + 2e− + 2H+ (3)

The voltammograms of complexes (1–3) (see Figures 4 and S16, Supporting Information) exhibit two quasi-reversible processes that were assigned to R−NO2/R−NO2− (peaks Ic and Ia), eq 4. The Eplc values indicate that the formation of R−NO2− is favored in (1–3) in comparison to the thiosemicarbazone ligands (Table 2).

An irreversible oxidation process (IVA) was also observed at 1137−1410 mV, which was attributed to the Au(I)−Au(II) oxidation, eq 5.

Electrochemistry Studies. Secnidazole and HL1−HL3 display a stable well-defined couple (system Ic/Ia), with Eplc in the −1120 to −1130 mV range, related to a one-electron transfer process attributed to the formation of a stable nitro radical anion (R−NO2). The voltammograms also exhibit a wave near −2000 mV, assigned to the generation of hydroxylamine (R−NOH) and, in some cases, an irreversible oxidation close to −500 mV, assigned to the formation of nitroso (R−NO) species (see eqs 1–3).

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Figure 4. Cyclic voltammogram of [Au(HL1)P(CH2CH3)3]PF6 (1) at 250 mV s−1. The red line shows a short sweep with the isolated RNO2/R−NO2− couple.

Table 2. Cyclic Voltammetry Parameters Relative to the R−NO2/R−NO2− and AuI/AuII Processes versus the Ag/AgCl, Cl− (3.0 M) Reference Electrode; 250 mV s−1

| compound | Eplc (mV) | ΔEp (mV) | Ep/a (mV) | Eplc (mV) |
|----------|----------|----------|-----------|-----------|
| HL1      | 1120     | 83       | 0.83      |           |
| [Au(HL1)P(CH2CH3)3]PF6 (1) | −978 | 133 | 0.82 | 1173 |
| HL2      | 1125     | 141      | 0.92      |           |
| [Au(HL2)P(CH2CH3)3]PF6 (2) | −1065 | 92 | 0.73 | 1410 |
| HL3      | 1130     | 98       | 0.85      |           |
| [Au(HL3)P(CH2CH3)3]PF6 (3) | −987 | 93 | 0.66 | 1137 |

The Epfc for complexes (1–3) were found in the - (978−1065) mV range, while for the thiosemicarazone ligands, these values were in the - (1120−1130) mV range, indicating that reduction of the nitro group is favored in the complexes.

Cytotoxic Activity. Taking into consideration that the nitroimidazole pharmacophoric group could, in principle, undergo reduction under anaerobic conditions resulting in the release of ROS that provokes DNA and protein damage, the cytotoxic effects of the compounds under study were evaluated against HCT-116 (colorectal cancer) cells under normoxia and hypoxia conditions and against HEK-293 (human embryonic kidney) nonmalignant cells in normoxia (see Table 3).

Although ligands HL1−HL3 and complexes (1–3) were designed to undergo a selective reduction of the nitro group under hypoxia, HL1−HL3 were not active against HCT-116 cells under hypoxia conditions. However, while HL3 was inactive against all of the tested cell lineages under normoxia.

Table 3. Cytotoxic Activity (IC50, μM) and Selectivity Indexes (SI) of the Compounds on HCT-116 and HEK-293 Cells

| compound | normoxia | hypoxia | normoxia | hypoxia |
|----------|----------|---------|----------|---------|
| HL1      | 5.6 ± 1.7 | 0.8     | >100     | ND      |
| HL2      | 4.4 ± 1.9 | 1       | >100     | ND      |
| HL3 >100 | ND       | >100    | >100     | ND      |
| 1        | 1.2 ± 0.3 | 3.7     | 5.7 ± 2.5 | 0.8     |
| 2        | 11.3 ± 1.7 | 1.1     | 3.5 ± 0.9 | 3.7     |
| 3        | 6.9 ± 2.4 | 1.3     | 6.0 ± 2.2 | 1.5     |
| [AuPEt3Cl] | 8.9 ± 2.1 | 0.4     | 5.2 ± 0.7 | 0.6     |
| TPZ      | 45.9 ± 7.2 | 0.6     | 7.2 ± 1.1 | 4       |

IC50 concentration that reduced 50% of cell proliferation; >100: at the highest concentration tested (100 μM), inhibition of 50% of cell viability was not verified and hence IC50 is above that value. SI: selectivity indexes (IC50 HEK-293/IC50 HCT-116 normoxia and hypoxia); ND: not determined, since the compound was inactive in the experimental conditions.
and hypoxia conditions, HL1 and HL2 were active against HCT 116 and HEK-293 cells under normoxia but not under hypoxia conditions. The values of log P are 1.06 (HL1), 1.39 (HL2) and 3.24 (HL3), indicating that HL3 is probably too lipophilic to reach the target.

As shown in Table 2, HL1–HL3 exhibit very unfavorable reduction potentials, and this might be the reason why they did not show cytotoxic activity under hypoxia. Upon coordination to gold(I), the reduction potentials become less negative so that complexes (1–3) and the triethylphosphinegold(I) precursor were active against both cell lineages in normoxia as well as under hypoxia conditions. Complex (1) was revealed to be active against HEK-293 and HCT-116 cells, being slightly more active against HCT-116 cells in normoxia than under hypoxia. Complex (3) was active against both cell lineages, being as active in normoxia as in hypoxia against the malignant HCT-116 cells. Since HL3 was inactive against all cell lineages, the cytotoxic effects of its organogold(I) complex (3) are probably due to the presence of the triethylphosphinegold(I) precursor, which shows cytotoxic effects against HCT-116 cells with IC50 values similar to those of complex (3), being more cytotoxic than 3 against HEK-293 cells.

In contrast, complex (2) proved to be more active against HCT-116 in hypoxia than in normoxia conditions, with the selectivity index, calculated as $SI = IC_{50\text{HEK-293}}/IC_{50\text{HCT-116}}$ hypoxia, equal to 3.7, similar to the value obtained for the control drug tirapazamine (TPZ, SI = 4). Interestingly, [AuP(CH3CH2)3Cl] also proved to be slightly more active against HCT-116 cells in hypoxia but its selectivity index was much lower, $SI = 0.6$.

The electrochemical behaviors of HL1–HL3 are very similar, as are the behaviors of their triethylphosphinegold(I) complexes (1–3), although the compounds showed distinct cytotoxic activities. The behavior of complex (2), however, deserves interest, since it was significantly more active against HCT-116 cells under hypoxia than under normoxia conditions and, hence, in this case, selective reduction of the nitro group in a low oxygen pressure milieu might operate, while in the remaining compounds, other modes of action probably occur.

In fact, complex (2) exhibited a selective index similar to that of tirapazamine, an experimental anticancer drug that is only activated in hypoxic areas of solid tumors. Hypoxia-activated prodrugs such as nitroimidazoles, nitrobenzenes, and $N$-oxides are inert prodrugs that diffuse through the extravascular tissue to hypoxic sites before their activation by means of a bio-reductive process. The common mode of action within these classes is the mono-electronic reduction of the bio-reducible prodrug by a reductase, with the formation of a radical anion. Under hypoxia conditions, the radical anion undergoes further reduction to an active agent that provokes cellular toxicity, fragments, or rearranges to produce a reactive species or activated drug.

In the presence of oxygen, this radical is reoxidized, releasing superoxide, which is easily processed by the cell-protective systems. This “futile” redox cycling results in low amounts of the prodrug radical in aerobic cells, leading to hypoxia-selective cell killing. Inhibition of drug reduction by oxygen through the redox cycling process was initially shown for nitro compounds and was later demonstrated to be responsible for the hypoxia-selective cytotoxicity of nitroimidazoles.

A common characteristic of the bio-reductive nitro-containing prodrugs presently in development is that their active metabolites are DNA-reactive cytotoxins. Hence, a plausible hypothesis is to explain the cytotoxic activity of complex (2) against HCT-116 cells under hypoxia may involve its bio-reduction with the formation of the toxic nitro anion radical NO2•. In fact, evidence for the formation of the nitro anion radical in a cell-free medium was obtained from the electrochemical studies of this complex. In addition, we previously demonstrated that the same 5-nitroimidazole ligands and their copper(II), silver(I), and bismuth(III) complexes were inactive against Gram-positive and -negative aerobic bacteria but proved to be active against anaerobic bacterial strains, suggesting reduction of the nitro group under low oxygen concentrations with production of the toxic nitro anion radical to be part of their mode of action.

Although complexes (1) and (3) showed electrochemical behaviors similar to that of complex (2), being also able to form the nitro anion radical in a cell-free medium, the lack of hypoxia selectivity of (1) and (3) might be related to distinct intrinsic sensitivities of their radical anion to oxygen, which influence the kinetic balance between the forward reactions and the back reaction with oxygen.

Taking into consideration the hypoxia-selective cytotoxicity of complex (2) and the resistance of tumor cells to radiotherapy and to most anticancer drugs in hypoxic regions, further investigations on this complex are of utmost relevance. In fact, gold compounds have been suggested as a potential alternative to platinum-based chemotherapeutic agents for colon tumor treatment.

**CONCLUSIONS**

The foregoing results suggest that coordination of the secnidazole-derived (E)-N-methyl-2-[(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ylidene]hydrazinecarbothioamide (HL2) to triethylphosphinegold(I) as in complex (2) was a valuable strategy for improving selectivity against HCT-116 cancer cells under hypoxia conditions. Indeed, since complex (2) proved to be as selective as the bio-reducible control drug tirapazamine, and considering that this complex contains the secnidazole and triethylphosphinegold(I) structural motifs, which are already used in the clinics, it deserves to be further investigated for its antitumor effects in vivo and its mechanism of action.

**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. A Digimed model DM3 conductivity bridge was used for molar conductivity measurements. Infrared spectra (4000–400 cm$^{-1}$) were recorded on a PerkinElmer Fourier transform infrared (FT-IR) Spectrum GX spectrometer using KBr pellets. NMR spectra were obtained with a Bruker DPX-400 Advance (400 MHz) and a Bruker DPX200 (200 MHz) spectrometer using DMSO-$d_6$ as the solvent and TMS as the internal reference.

Cyclic voltammetry experiments were performed at room temperature with a three-electrode cell (with a volumetric capacity of 10 mL) in an Autolab type PGSTAT30 equipment using Nova 2.1.3 software. A Metrohm glassy carbon electrode was used as the working electrode, and the auxiliary electrode was a platinum wire. $Ag/AgCl$, $Cl^-$ (3.0 M) was employed as the reference electrode. The glassy carbon electrode was
previously fine-polished with a 0.3 μm alumina slurry on a polishing felt for 5 min. Solutions were prepared in spectroscopic dimethylformamide (DMF) containing 1 mM analyte and 0.1 M tetrabutylammonium perchlorate (TBAP) as supporting electrolyte. Before recording the voltammograms, the test solution was thoroughly purged with high-purity nitrogen to remove any traces of dissolved oxygen. Cyclic voltammograms were recorded in the 2.00 to −2.00 V potential range using a scan rate of 250 mV s⁻¹.

**Syntheses of Triethylphosphinegold(I) Complexes (1−3).** Complexes (1−3) were obtained by reacting [AuP(CH₂CH₃)₃]Cl with the desired thiosemicarbazone in a 1:1 M/L ratio. Hence, 0.5 mmol of [AuP(CH₂CH₃)₃]Cl was dissolved in a mixture of 6 mL of methanol and 6 mL of dichloromethane, followed by the addition of 0.7 mmol of KPF₆ and a slight excess (0.7 mmol) of the appropriate thiosemicarbazone. The reaction mixture was kept under reflux and stirring in the dark for 3 h (see Figure 1). The yellow solid formed after solvent evaporation was filtered off and washed with diethylether, and dried under reduced pressure. The complexes were characterized by means of microanalyses and their de 1H, 13C, DEPT 135, COSY, 1H, 13C NMR spectroscopy, empirical formula C₉H₁₄N₆O₂SC₁₄H₁₈N₆O₃S.

| compound  | HL2         | HL3         |
|-----------|-------------|-------------|
| empirical formula | C₆H₇N₄O₃S  | C₆H₉N₃O₂S  |
| formula weight (g mol⁻¹) | 270.32      | 350.40      |
| wavelength (Å) | 0.71073     | 0.71073     |
| crystal system | triclinic    | orthorhombic |
| space group | P ʻ           | Pbca        |
| temperature (K) | 298(2)      | 298(2)      |
| a (Å) | 8.1536(4)    | 15.3965(5)  |
| b (Å) | 8.7320(6)    | 13.6200(4)  |
| c (Å) | 8.9348(5)    | 16.0574(4)  |
| α (deg) | 89.139(5)   | 90          |
| β (deg) | 81.378(4)   | 90          |
| γ (deg) | 68.290(6)   | 90          |
| V (Å³) | 642.57(7)    | 3362.70(17) |
| Z | 2           | 8           |
| density (calculated) (g cm⁻³) | 1.397       | 1.384       |
| crystal size (mm³) | 0.241 × 0.214 × 0.125 | 0.723 × 0.468 × 0.425 |
| F(000) | 284.0        | 1472.0      |
| absorption coefficient (mm⁻¹ Å⁻¹) | 0.257       | 0.219       |
| T_{min}/T_{max} | 0.614 /1 | 0.475 /1 |
| 2θ range for data collection (deg) | 3.18 to 27.033 | 3.214 to 29.291 |
| reflections collected | 14624 | 4639 |
| independent reflections | 11 169 | 3439 |
| number of parameters | 164 | 217 |
| final R indexes | R₁ = 0.0659 wR₂ = 0.1960 | R₁ = 0.0489 wR₂ = 0.1538 |
| goodness-of-fit on F² | 1.086 | 0.0678 |
| goodness-of-fit on F² | 1.127 | 1.027 |
| Δρ_{max} and Δρ_{min} | 0.384 and −0.396 | 0.309 and −0.256 |

For compounds were collected using an Agilent SuperNova diffractometer with Mo Kr (λ = 0.7073 Å) radiation at room temperature (298 K). The data collection, cell refinements, and data reduction were performed using CRYSALISPRO software. The structures were solved by direct methods and refined using SHELXL-2018/3. All nonhydrogen atoms were
refined with anisotropic thermal parameters. H atoms connected to carbon were placed in idealized positions and treated by a rigid model, with Uiso(H) = 1.2Ueq(C), while H atoms from NH groups were obtained directly by difference maps and fixed with a N–H distance around 0.88 Å and Uiso(H) = 1.2Ueq(N). CCDC 1949760 and 1949761 contain the supplementary crystallographic data for HL2 and HL3, respectively. The figures were drawn using ORTEP-3 for Windows and Mercury.37

Evaluation of the Cytotoxic Effects of the Compounds. Colorectal carcinoma (HCT-116) and human embryonic kidney (HEK-293) cells were donated by Dr. Marcel Leist, University of Konstanz/Germany, and maintained in Dulbecco’s modified Eagle’s medium (DMEM) medium (Sigma Aldrich) with 10% fetal serum (GIBCO BRL, Grand Island, NY) and enriched with a 1% antibiotic solution (100 IU/mL penicillin and 100 μg/mL streptomycin (GIBCO BRL, Grand Island, NY)). Cytotoxic activity was based on the mitochondrial reduction rate of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan crystals.35 HCT-116 and HEK-293 cells were seeded at the density of 5 × 103 cells per well (96-well plate) and preincubated overnight for stabilization under hypoxia conditions (1% O2, 5% CO2, 94% N2 at 37 °C) and normoxia (5% CO2, 95% atmospheric air at 37 °C) as described by Harrison and co-workers.39 After stabilization, cells were incubated for 72 h with the compounds and with tirapazamine (TPZ) as a positive control, using seven serial 1:5 dilutions (100–0.0064 μM), under conditions of hypoxia and normoxia. The IC50 (inhibitory concentration of 50% cell proliferation) was determined by nonlinear regression using GraphPad Prism Version 5.01 software (GraphPad Software Inc., La Jolla, CA). All compounds were tested in four independent experiments performed in triplicate and the solvent (DMSO 0.5%) was used as the control. The results were expressed as mean ± standard deviation of the mean of the percentage of viability in relation to the negative control (DMSO, 0.5%), calculated as follows: percentage of cell viability (%) = [(OD of treated sample/OD negative control) x100].38 Selectivity indexes (SI) of the compounds were also evaluated using the ratio between the 50% inhibitory concentration of cell viability for nonmalignant and tumor cells, SI = IC50(HEK-293)/IC50(HCT-116) under normoxia and hypoxia conditions.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03778.

X-ray crystallographic data for HL2 (CIF)
X-ray crystallographic data for HL3 (CIF)

1H and 13C NMR and FT-IR spectra of HL1-HL3 and their organometallic gold(I) complexes [Au(HL1)P(CH2CH3)3]PF6 (1), [Au(HL2)P(CH2CH3)3]PF6 (2), and [Au(HL3)P(CH2CH3)3]PF6 (3); electrochemical data (cyclic voltammograms) of complexes (1–3) and X-ray crystallographic data for HL2 and HL3 (PDF)

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Notes
The authors declare no competing financial interest.
CCDC reference numbers 1949760 and 1949761 contain the supplementary crystallographic data for HL2 and HL3. These data can be obtained free of charge from the CCDC via http://www.ccdc.cam.ac.uk/conts/retrieving.html.

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