Enhanced Bactericidal Effects of Pyrazinamide Toward *Mycobacterium smegmatis* and *Mycobacterium tuberculosis* upon Conjugation to a {Au(I)-triphenylphosphine}+ Moiety

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As part of the quest for new gold drugs, we have explored the efficacy of three gold complexes derived from the tuberculosis drug pyrazinamide (PZA), namely, the gold(I) complex \([\text{Au(PPh}_3\text{)(PZA)}]\)OTf (1, OTf = trifluoromethanesulfonate) and two gold(III) complexes \([\text{Au(PZA)Cl}_2]\) (2) and \([\text{Au(PZO)Cl}_2]\) (3, PZO = pyrazinoic acid, the metabolic product of PZA) against two mycobacteria, *Mycobacterium tuberculosis* and *Mycobacterium smegmatis*. Only complex 1 with the \{Au(PPh$_3$)}+ moiety exhibits significant bactericidal activity against both strains. In the presence of thiols, 1 gives rise to free PZA and \{Au(PPh$_3$)}-thiol polymeric species. A combination of PZA and the \{Au(PPh$_3$)}-thiol polymeric species appears to lead to enhanced efficacy of 1 against *M. tuberculosis*.

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The success of auranofin and the rapid increase in drug-resistant pathogens prompted a surge of new research into gold compounds and their broad use as antiarthritic, antimicrobial, anticancer, and antifungal agents. Gold(III) complexes, sharing the same geometrical shape as the well-known cancer therapeutic cisplatin, have been evaluated for their anticancer effect and often show increased activity with no cross-resistance to cisplatin. Antimicrobial gold(I) and (III) complexes have been investigated for their potency against numerous strains of Gram-positive and Gram-negative bacteria showing promising results. So far, research in this area has implied that ligand structures of the gold complexes play important roles in both their efficacy and toxicity. In particular, manipulation of ligand structures was shown to affect the passage of the therapeutics across hydrophobic membranes of target cells to a significant extent. An important class of gold(I) compounds, those with triphenylphosphine as ligands, is emerging as effective therapeutics against bacterial infection and cancer (Figure 1). The results of our previous work on cationic gold(I) triphenylphosphine complexes have implied that the highly reactive and lipophilic \{Au(PPh$_3$)}+ unit plays an important role in their antibacterial activity. Although gold(I) complexes have been studied for their anticancer and antibacterial effectiveness, to our knowledge only two gold(I) complexes with the \{Au(PPh$_3$)}+ unit have so far been evaluated for their antimycobacterial behavior.

**ABSTRACT:** As part of the quest for new gold drugs, we have explored the efficacy of three gold complexes derived from the tuberculosis drug pyrazinamide (PZA), namely, the gold(I) complex \([\text{Au(PPh}_3\text{)(PZA)}]\)OTf (1, OTf = trifluoromethanesulfonate) and two gold(III) complexes \([\text{Au(PZA)Cl}_2]\) (2) and \([\text{Au(PZO)Cl}_2]\) (3, PZO = pyrazinoic acid, the metabolic product of PZA) against two mycobacteria, *Mycobacterium tuberculosis* and *Mycobacterium smegmatis*. Only complex 1 with the \{Au(PPh$_3$)}+ moiety exhibits significant bactericidal activity against both strains. In the presence of thiols, 1 gives rise to free PZA and \{Au(PPh$_3$)}-thiol polymeric species. A combination of PZA and the \{Au(PPh$_3$)}-thiol polymeric species appears to lead to enhanced efficacy of 1 against *M. tuberculosis*.

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Recent efforts in drug discovery have demonstrated that combination of two antipathogenic moieties in one chemotherapeutic often leads to higher overall efficacy.\textsuperscript{11−16} It is evident that such a design provides a dual mechanism of action and potentially increased the effect compared to that of the ligand or the metal center on its own. Along this line, we have reported Au(I) and Ag(I) complexes of benzothiazoles that exhibit high antibacterial activity against \textit{Acinetobacter baumannii}, \textit{Pseudomonas aeruginosa}, and \textit{Staphylococcus aureus}. Success in these cases prompted us to explore the possibility of designing new gold(I) complexes comprising the \{Au(PPh$_3$)$_2$\}\textsuperscript{+} moiety and the known TB drug pyrazinamide (PZA). PZA shortens the treatment duration for TB to a considerable extent although large oral quantities are needed for such effect.\textsuperscript{17} We therefore decided to combine PZA with the \{Au(PPh$_3$)$_2$\}\textsuperscript{+} unit and study the synergistic effects (if any) on \textit{M. tuberculosis} and the possibility of use of lower doses of PZA.

Herein, we report the syntheses and characterization of one gold(I) complex [Au(PPh$_3$)\textsubscript{3}(PZA)]\textsuperscript{+} (1, OTf = trifluoromethanesulfonate) and two gold(III) complexes [Au(PZA)\textsubscript{2}Cl\textsubscript{2}] (2) and [Au(PZO)\textsubscript{2}Cl\textsubscript{2}] (3, PZO = pyrazinoic acid, the metabolic product of PZA). The antimycobacterial properties of these complexes have been evaluated on both \textit{M. tuberculosis} and \textit{Mycobacterium smegmatis}.

**EXPERIMENTAL SECTION**

**General.** All reagents and solvents were of commercial grade and used without further purification. Fourier-transform infrared (FT-IR) spectra were obtained using a PerkinElmer Spectrum One spectrophotometer. $^1$H, $^{13}$C, $^{31}$P, and $^{19}$F nuclear magnetic resonance (NMR) spectra were recorded using a Varian Unity 500 MHz instrument at 298 K.

**Syntheses.** [Au(PPh$_3$)\textsubscript{3}(PZA)]\textsuperscript{+} (1). A solution of 72.0 mg (0.28 mmol) of silver trifluoromethanesulfonate (OTf) in 10 mL of methanol was added to a solution of 138.8 mg (0.28 mmol) of a sharply defined Au(PPh$_3$)$_3$ complex. The mixture was stirred at room temperature for 24 h. A black precipitate formed, resulting in a yellow solution. After filtration and washing with methanol, the complex was dried under vacuum. The complex was characterized by analytical and spectral techniques.

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Table 1. Crystal Data and Structure Refinement Parameters for 1·H$_2$O, 2, and 3

|                  | 1·H$_2$O | 2 | 3 |
|------------------|----------|---|---|
| **Formula**      | C$_2$H$_9$AuN$_3$OP$_2$CF$_3$O$_2$S·H$_2$O | C$_3$H$_7$AuCl$_3$N$_3$O$_5$ | C$_5$H$_3$AuCl$_2$N$_3$O$_2$ |
| **D$_{cal}$ (g cm$^{-3}$)** | 1.835 | 3.135 | 3.013 |
| **μ (mm$^{-1}$)** | 5.62 | 18.4 | 17.65 |
| **Formula weight** | 749.44 | 389.98 | 390.96 |
| **Color** | colorless | yellow | yellow |
| **Shape** | block | plate | plate |
| **T (K)** | 298 | 298 | 298 |
| **Crystal system** | triclinic | triclinic | orthorhombic |
| **Space group** | P1 | Pbca | Pbca |
| **a (Å)** | 7.0334 (10) | 6.6857 (10) | 7.2868 (6) |
| **b (Å)** | 12.6524 (16) | 7.2848 (11) | 14.4003 (12) |
| **c (Å)** | 15.688 (2) | 8.9057 (13) | 16.4259 (13) |
| **α (°)** | 79.435 (4) | 94.664 (2) | 90 |
| **β (°)** | 81.214 (5) | 106.878 (2) | 90 |
| **γ (°)** | 89.464 (4) | 91.649 (2) | 90 |
| **V (Å$^3$)** | 1356.0 (3) | 413.06 (11) | 1723.6 (2) |
| **Z** | 2 | 8 | 8 |
| **Wavelength (Å)** | 0.71073 | 0.71076 | 0.71076 |
| **Radiation type** | Mo Kα | Mo Kα | Mo Kα |
| **2θ$_{max}$ (°)** | 52.8 | 49.6 | 49.6 |
| **2θ$_{max}$ (°)** | 124.132 | 4259 | 15.095 |
| **Independent refl.** | 5469 | 1395 | 1462 |
| **Reflections used** | 5027 | 1381 | 1261 |
| **R$_{int}$** | 0.025 | 0.019 | 0.044 |
| **Goof** | 1.14 | 1.2 | 1.08 |
| **wR$_2$** | 0.122 | 0.043 | 0.067 |
| **R$_1$** | 0.044 | 0.017 | 0.024 |

$^a$GOF = ($\sum_i[w(F_{o,i}^2 - F_{c,i}^2)^2]/(N_o - N_v)$$)^{1/2}$ (N$_o$ = number of observations, N$_v$ = number of variables). $^b$w = [($\sum_i[w(F_{o,i}^2 - F_{c,i}^2)^2]/\sum|F_{o,i}|\sum|F_{c,i}|$].
mmol) of chloro(triphenylphosphine)gold(I) dissolved in 15 mL of chloroform. Immediately, a suspension of AgCl was formed and the mixture was stirred for 30 min at room temperature and then filtered through a bed of celite. To the filtrate, a solution of 34.4 mg (0.28 mmol) of PZA in 15 mL of methanol/chloroform (1:1) was added, and the mixture was stirred for 18 h at room temperature. The flask was covered with an Al foil to protect the reaction mixture from ambient light. Next, the solvent was removed in vacuo, and the solid was washed with diethyl ether to obtain a white solid (112.1 mg, 54%). A solution of the solid in chloroform was layered with hexanes and stored in the fridge. X-ray quality crystals of 1 were obtained after 1 week. Anal. calc. for $\text{C}_{24}\text{H}_{30}\text{AuN}_{3}\text{O}_{5}\text{PSF}_5$: C, 39.41; H, 2.76; N 5.74; found: C, 39.35; H, 2.79, N, 5.68. IR (KBr, cm$^{-1}$): 3435 (m), 3309 (m), 1690 (s), 1438 (m), 1262 (s), 1167 (m), 1031 (m), 693 (m). 1H NMR (CD$_3$CN, ppm): 9.40 (b, 1H), 9.03 (b, 1H), 8.96 (b, 1H), 7.69–6.58 (m, 15H). 13C (CD$_3$CN, ppm): 171.27, 152.97, 149.89, 141.50, 134.26, 134.16, 132.54, 132.52, 129.64, 129.55, 127.55, 127.03. 13P (MeOD, referenced to PPH$_3$), 34.88.

$[\text{Au}(\text{PZA})\text{Cl}]_2$ (2). A batch of 32.5 mg (0.26 mmol) of PZA was dissolved in 2 mL of water and the solution was added dropwise to a solution of 99.7 mg (0.26 mmol) of potassium tetrachloroaurate in 1.75 mL of water at room temperature with stirring. A yellow solid appeared quickly, and the mixture was stirred for 18 h at room temperature. The yellow solid was filtered through a bed of celite. To the filtrate, a solution of 34.4 mg (0.28 mmol) of PZA in 15 mL of methanol/chloroform (1:1) was added, and the mixture was stirred for 18 h at room temperature. The yellow solid was collected on a Bruker APEX II single-crystal X-ray diffractometer (PHOTON 100 detector) with graphite monochromated Mo Kα radiation ($\lambda = 0.71073 \text{ Å}$) by the $\omega$-scan technique in the range 5.8 $\leq \theta \leq$ 53 for (1), 7 $\leq \theta \leq$ 50 for (2), and 6.2 $\leq \theta \leq$ 50 for (3) (Table 1). All data were corrected for Lorentz and polarization effects.\textsuperscript{18} All of the structures were solved with the aid of the SHELXT program using intrinsic phasing.\textsuperscript{19} The structures were then refined by a full-matrix least-squares procedure on $F^2$ by SHELX.\textsuperscript{20} All nonhydrogen atoms were refined anisotropically. All hydrogen atoms were included in calculated positions. The absorption corrections are done using SADABS.\textsuperscript{19} Calculations were performed using the OLEX2\textsuperscript{21} and SHELXTL\textsuperscript{22} (V 6.14) program package.

**Mycobacterial Studies.** *M. smegmatis.* Middlebrook 7H9 liquid medium\textsuperscript{23} was inoculated from a frozen stock of *M. smegmatis* and grown overnight to an optical density at 600 nm (OD$_{600}$) of 1. Stock solutions of test compounds in acetone (0.02–0.1 mM) were prepared, and batches of 20 µL of such solutions were added to 250 µL of the bacterial suspensions in 1.73 mL of 7H9 media in 5 mL culture tubes. The tubes were incubated at 37 °C for 18 h. The MIC values were then determined by reading the OD$_{600}$ of the suspensions with different concentrations of the test compounds. To ensure that no viable bacteria remained in such tubes was confirmed as follows. Aliquots of 100 µL of the suspensions were added to fresh 7H9 media (1 mL) and incubated at 37 °C for 18 h. In all cases, no bacteria growth was observed. The MIC results are summarized in Table 3, and all concentrations were performed in triplicate.

*M. tuberculosis.* A stock culture of *M. tuberculosis* was prepared by inoculation of a 1 mL frozen stock into 50 mL of Middlebrook 7H9 liquid medium supplemented with 10% (v/v) OADC enrichment (BBL Middlebrook OADC, 212351), 0.5% (v/v) glycerol, and 0.05% (w/v) Tween 80 (P1754, Sigma-Aldrich) in a roller bottle. Cells were grown to an OD$_{600}$ of 1.0 to begin the experiments. The culture was then diluted down to a target OD$_{600}$ of 0.5 (final OD reading = 0.67). Aliquots of 100 µL of 8 mM test compound solutions in acetone were added to batches of 10 mL of the culture suspension (final concentration of the test compounds = 80 µM in 1% acetone) and the tubes were then incubated at 37 °C. After 24 h incubation, the OD$_{600}$ values were recorded. Triplicates were run with each test compound, and the results are shown in Figure 5.

**RESULTS AND DISCUSSION**

**Synthesis.** Complex 1 was synthesized by first displacing the chloride ligand from [ClAu(PPh$_3$)$_3$] with the aid of Ag(OTf) and introducing PZA as the second ligand. Single-crystal analysis of 1 (Figure 2) revealed PZA as a monodentate N-donor ligand.\textsuperscript{24} The IR spectrum of the analogous [AuCl$_3$(pyrazine)] complex.\textsuperscript{25} Slow evaporation of the methanolic solution of this precursor complex eventually afforded complex 2. Comparison of the IR spectra of the precursor complex and 2 reveals different ν$_{\text{CO}}$ frequencies (1704 and 1660 cm$^{-1}$, respectively) corresponding to the amide CO group of PZA. Because the ν$_{\text{CO}}$ of the precursor complex is close to the ν$_{\text{CO}}$ value of free PZA (1711 cm$^{-1}$), we
believe that in this complex the PZA ligand is bound to the Au(III) center in a monodentate fashion (as shown in Scheme 1). In complex 2, the PZA ligand is bonded as a bidentate ligand (Figure 3) with the deprotonated amide group. Elimination of HCl from the precursor complex leads to formation of complex 2 in methanolic solution upon long evaporation (Scheme 1). Due to the relatively quick reaction time (10 min), the precursor material presumably precipitates outuasthetikinetically favored species while 2 is the thermodynamically favored species obtained after recrystallization from methanol. The assignment is further supported by the fact that while the precursor complex exhibits two amide NH peaks in its NMR spectrum (much like free PZA) in CD$_2$CN, complex 2 displays only one NH peak in its NMR spectrum in the same solvent (Figure 4). Also the precursor complex, like [AuCl$_3$(pyrazine)], readily loses the N-donor ligand in dimethyl sulfoxide (DMSO)-d$_6$ (as evidenced by NMR spectra). We hypothesize that in the precursor complex, PZA is bound to the Au(III) center at the N atom meta to the carboxamide group (as observed in complex 1) simply because this N center is the most basic site of the PZA molecule. Conversion of the precursor complex to 2 is accelerated by the addition of NaHCO$_3$ in a 1:1 MeOH/water reaction mixture, a step that pushes the reaction shown in Scheme 1 to the right.

PZO, the purported metabolic product actually responsible for the drug action of PZA, also binds the Au(III) center as a bidentate ligand (Figure 3, bottom panel). Addition of excess deprotonated PZO to KAuCl$_4$ in the aqueous medium affords complex 3 in high yield.

**Structures.** Structures of 1−3 were characterized by X-ray crystallography and the perspective views (with atom labeling schemes) are shown in Figures 2 and 3, while the structure refinement parameters and selected bond distances and angles are listed in Tables 1 and 2, respectively. For the sake of comparison of the metric parameters, the two Au(III) complexes (2 and 3) are shown in Figure 3. As evident from their crystal structures, the Au(I) complex 1 exhibits a linear

![Figure 2. Structure of 1 with water and OTf anion omitted for clarity. Ellipsoids are shown at the 50% probability level.](image1)

![Figure 3. Crystal structures of 2 (top) and 3 (bottom) with thermal ellipsoids at 50% probability.](image2)

![Scheme 1. Suggested Ligand Binding Mode Rearrangement from the Precursor Complex (Left) to 2](image3)

![Figure 4. $^1$H NMR (in CD$_2$CN) spectra of 2 (top), the precursor compound (middle), and PZA (bottom).](image4)

Table 2. Selected Bond Lengths and Angles for 1·H$_2$O, 2, and 3

| Bond Lengths and Angles | 1·H$_2$O | 2 | 3 |
|-------------------------|----------|---|---|
| Au(I)−P(1)              | 2.2432 (18) |   |   |
| Au(I)−N(1)              | 2.082 (6)  |   |   |
| Au(I)−N(3)              | 1.985 (4)  | 2.016 (6) |   |
| Au(I)−N(2)              | 2.042 (4)  | 2.250 (2) |   |
| Au(I)−Cl(1)             | 2.2571 (12)| 2.250 (2) |   |
| Au(I)−Cl(2)             | 2.2879 (12)| 2.2510 (17)|   |
| Au(I)−O(2)              | 1.998 (4)  |   |   |
| N(1)−Au(I)−P(1)         | 177.8 (2)  |   |   |
| N(3)−Au(I)−Cl(1)        | 93.22 (12) |   |   |
| N(2)−Au(I)−N(3)         | 80.51 (16) |   |   |
| Cl(1)−Au(I)−Cl(2)       | 90.75 (5)  | 90.68 (7) |   |
| N(2)−Au(I)−O(2)         | 95.63 (11) | 95.57 (14) |   |
| O(2)−Au(I)−Cl(1)        | 82.7 (2)   |   |   |
|                         | 91.01 (16) |   |   |
coordination, while the two Au(III) complexes are square planar. In the structure of I, there is one molecule of water in the asymmetric unit, while the other two structures contain no solvent of crystallization. The N(1)−Au(1)−P(1) angle in I deviates slightly from linearity with an angle of 177.8(2)°. The Au−N(pyridine) (2.081(7) Å) bond is shorter than Au−P (2.244(2) Å) bond as expected. Similar bond lengths and angles are observed in other known structures of Au(I) complexes of [(N-bound ligand)Au(PPh3)]-type.11,14 The three nitrogen atoms on PZA potentially allow for three different binding modes to the {Au(PPh3)}⁺ unit in 1; however, the least sterically hindered and most basic N of PZA shows preference to the metal center, as shown in Figure 1.26

Au(III) complexes 2 and 3 are both distorted square planar and composed of the PZA/PZO ligand bound as a bidentate ligand. The square planar geometries of 2 and 3 deviate noticeably from planarity with N(2)−Au(1)−N(3) and N(2)−Au(1)−O(2) angles of 80.51(16) and 82.7(2)°, respectively. The Cl(1)−Au(1)−Cl(2) angles of both structures deviate only slightly from the perfect right angle values 90.75(5) and 90.68(7)°, respectively. As expected, the deprotonated Au(1)−N(3) or Au(1)−O(2) of 2 and 3 are significantly shorter than the Au(1)−N(2) bonds shown in Table 2. Bond lengths and angles are in agreement with similar known structures of Au(III)-picolinate and picolinic acid derivatives.27,28

In 2, the equatorial plane comprised of Au(1), N(2), N(3), Cl(1), Cl(2) atoms is fairly planar with a mean deviation of 0.041(3) Å, while the corresponding plane in 3 (comprised of Au(1), O(2), N(2), Cl(1), Cl(2) atoms) is highly planar with a mean deviation of 0.011(3) Å. The central metal atom in 2 and 3 is deviated from these planes by 0.009(3) and 0.022(3) Å, respectively. The two chelate planes formed by the bidentate PZA and PZO ligands along with Au(III) centers in 2 (Au(1), N(2), N(3), C(1), and C(5)) and 3 (Au(1), N(2), O(2), C(1), and C(5)) exhibit minimal deviation from planarity (mean deviations, 0.011(3) and 0.019(3) Å for 2 and 3, respectively). The dihedral angles between the pyrazine ring and the five-membered chelate ring in 2 and 3 are 3.18(2) and 1.83(2)°, respectively. In an Au(III) complex with picolinate as a ligand, which structurally resembles closely to that of complex 2, the dihedral angle between the pyridine ring and the five-membered chelate ring is 3.6(2)°, which is close to the corresponding value noted for 2.27 Moreover, the mean deviations of the chelate ring are similar to those in 2. However, a reported Au(III) complex with a picolinic acid derivative as the ligand resembles structurally more to complex 3, and the dihedral angle between the pyridine ring and the five-membered chelate ring is 1.28(2)°.28

**Mycobacterial Activity.** Before studying the potential synergistic effects of PZA and gold on *M. tuberculosis*, the antimicrobial effects of the Au center alone were studied on *M. smegmatis*. This bacterium is in the same genus as *M. tuberculosis* and has 2000 genes highly conserved with the pathogen. Thus, *M. smegmatis* is an excellent model organism that is easy to work with, has a fast growth rate, and a relatively safer model.23,29 *M. smegmatis* is known to be naturally resistant to PZA and thus provides an opportunity to study the activity of the {Au(1)(PPh3)}⁺ and {Au(III)Cl3}⁺ moieties of 1−3. In the present work, the activities of 1−3, [ClAu(PPh3)], PZA, and a drug control isoniazid (INH) were tested against *M. smegmatis* in a normal growth environment and the results are summarized in Table 3. Under our conditions, we found the MIC to be 60 μM for 1 and [ClAu(PPh3)], while 2, 3, and PZA showed no activity up to 100 μM. Mycobacteria species are known to have thick, hydrophobic, and waxy membranes that prevent foreign substances from permeation more so than traditional Gram-positive and Gram-negative species.27,31 For this reason, the lipophilic {Au(PPh3)}⁺ unit in 1 might have been able to pass through this membrane and exert drug action. This conclusion is supported by the fact that cell digests from *M. smegmatis* cells exposed to 40 μM (below the MIC) of 1 exhibited strong inductively coupled plasma mass spectrometry (ICP-MS) signal(s) for gold. Also, the gold(III) species 2 and 3 with chloro ligands but no {Au(PPh3)}⁺ moiety were not active at similar concentrations.

With results from *M. smegmatis* study in hand, we proceeded to test the activity of complex 1 against *M. tuberculosis* in vitro along with [ClAu(PPh3)], PZA, and isoniazid (INH). The OD of *M. tuberculosis* was recorded after 24 h incubation with 80 μM of each compound in 1% acetone (Figure 5). Interestingly, 1 showed significant bactericidal activity (reduction in OD), while PZA on its own was only mildly bacteriostatic (OD less than the control but higher than day 0). The mild drug action of PZA against *M. tuberculosis* at the 80 μM concentration is expected since the higher concentration of PZA (up to 400 μM) and low pH (5.5) media are usually required to observe any effect on *M. tuberculosis* growth in vitro.32 The results shown in Figure 5 strongly suggest that the {Au(PPh3)}⁺ moiety of 1 augments the efficacy of PZA in vitro. The standard [ClAu(PPh3)] was specifically included in this study to determine if 1 would show increased activity compared to a compound with the {Au(PPh3)}⁺ moiety without PZA. Inspection of Figure 5 reveals that both 1 and [ClAu(PPh3)] exhibited the greatest reduction in OD at very similar average values of 0.345 and 0.387, respectively. Collectively, these results suggest that complex 1 could introduce a dose of PZA as well as the {Au(PPh3)}⁺ moiety in one combination and act

| Table 3. MIC (μM) Values for Activity Against *M. smegmatis* |
| --- |
| **compound** | **MIC (μM)** |
| 1 | 60 |
| 2 | >100 |
| 3 | >100 |
| [ClAu(PPh3)] | 60 |
| PZA | >100 |
| INH | 80 |

![Figure 5. *M. tuberculosis* OD_{600} values of the initial (day 0) and after 24 h (day 1) incubation with test compounds at 80 μM. Column C has no additional compound and acetone (Ace).](https://dx.doi.org/10.1021/acsomega.0c00071)
the anticancer effects has been hypothesized to play an important role in improved outcomes. Nevertheless, PZA has had a significant clinical impact on TB because it targets mostly nonreplicating bacilli, PZA exhibits slow or no bactericidal activity in vitro. This occurrence is likely the reason why no synergy was observed in our hands. The mechanism of action of the pro-drug PZA is not entirely understood, but most agree that the conversion of PZA to PZO within the bacilli is critical to the pro-drug PZA is not as active as PZA against M. tuberculosis. PZA has a broad range of activity highly dependent on the pH of the media and because it targets mostly nonreplicating bacilli, PZA exhibits slow or no bactericidal activity in vitro. This occurrence is likely the reason why no synergy was observed in our hands. Nevertheless, PZA has had a significant clinical impact on TB and research toward modifying PZA with the additional antimycobacterial moiety might lead to new and improved outcomes.

Solution studies. The results of $^1$H NMR studies confirm that 1–3 are stable in acetone, while 2 and 3 are also stable in acetonitrile. Complexes 2 and 3 are stable in aqueous acetone (90:10) for hours, while 1 slowly decomposes in such solutions (used in biological studies). However, inside biologically relevant environments, exposure to sulfur-containing biomolecules like glutathione or thioredoxin is expected. Gold(I) centers are soft Lewis acids, and it is well established that they prefer binding to soft Lewis bases like thiolate species. The binding of gold(I) species to biologically relevant thiols (such as glutathione) and enzymes with thiol-containing active sites (such as thioredoxin) has been observed and the exchange of N- and S-bound ligands occurs quickly compared to P-bound ligands. This exchange has been hypothesized to play an important role in the anticancer effects of [N−Au(PPh$_3$)$_3$] complexes as well as the antimycobacterial effects exhibited by auranozin. Disruption of redox homeostasis within the bacterial cell following binding of the gold unit to glutathione or thioredoxin has been suggested to be responsible for the drug action. Impairment of protein synthesis in bacteria has also been observed with auranozin treatment. In our previous work, we have shown that complexes with N-bound benzothiazole ligands and the [Au(PPh$_3$)$_3$]$^+$ moiety rapidly exchange with thiol species. In the present work, we checked the possibility of exchange between PZA and biologically relevant thiols in the presence of a thiol. We hypothesize that such a transformation will lead to the presence of both PZA and [Au(PPh$_3$)$_3$]$^+$ units, which will exert their own individual and potentially synergistic actions. In contrast, [ClAu(PPh$_3$)$_3$] does not appear to react with FTP and form any Au–thiol species (Figure 6A, middle panel). In the acidic (pH 6.2−4.5) macrophage compartment, replacement of Cl$^-$ by a thiol is highly unlikely. Thus, administration of 1 (compared to [ClAu(PPh$_3$)$_3$]) could be more reactive against M. tuberculosis residing within the macrophages in vivo. We have also employed a more biologically relevant thiole, namely, N-acetyl l-cysteine methyl ester, to check this interpretation. Addition of N-acetyl l-cysteine methyl ester to 1 resulted in the immediate appearance of a white precipitate (Figure 6B, right), but no such reaction was observed with [ClAu(PPh$_3$)$_3$] (Figure 6B, left). Collectively, these results suggest that formation of (S−Au(PPh$_3$)$_3$)-polymeric species with 1 within biological targets might promote uptake by host macrophages, similar to the uptake of Au nanoparticles and/or breakdown of cellular thiol-redox homeostasis. Since M. tuberculosis is either contained by macrophages or reside within them, this process could offer a more direct route to TB treatment in a host system. The (S−Au(PPh$_3$)$_3$)-polymeric species derived from 1, along with PZA, could cause more damage to the mycobacterium residing within the macrophages and thus increasing the efficacy of the treatment.

CONCLUSIONS

While Au complexes have been evaluated for their antimycobacterial effects previously, extensive literature search reveals only two other complexes containing the [Au(PPh$_3$)$_3$]$^+$ unit (Figure 7) in such testing. Also, in a previous study, the auranozin containing PEt$_3$ moiety has been shown to exert potent activity against M. tuberculosis. Between the two complexes with the [Au(PPh$_3$)$_3$]$^+$ unit, the one with the acridine moiety as a ligand (Figure 7, left) was screened against M. tuberculosis, while the other with sulfadiazine as a ligand (Figure 7, right) was tested against...
other members of the mycobacterium genus. The sulfadiazine–Au(PPh3) complex showed synergy of the sulfadiazine antibiotic with the {Au(PPh3)}+ unit. We expect that similar synergy between PZA and the {Au(PPh3)}+ moiety (arising from 1) could increase the efficacy of the treatment compared to PZA alone under in vitro conditions.

Current TB treatment regimens require four orally taken drugs (PZA, ethambutol, rifampicin, isoniazid) at high dosages for 6 months with the daily dose of PZA recommended at 30 mg/kg for at least 2 of those months. 17 Much of the difficulty of treatment for this disease arises from both the inherent thick, waxy mycomembrane (preventing foreign substances of treatment for this disease arises from both the inherent thick, waxy mycomembrane (preventing foreign substances from permeation) of M. tuberculosis and the success to survive within host macrophages. 30,31 Complexes like 1, as evidenced by our results, may react immediately with biologically relevant thiols to produce free PZA and {Au(PPh3)}–thiol polymeric species. The {Au(PPh3)}+ moiety of 1 may provide the lipophilic requirement to pass through the mycomembrane to exert its action, while a dose of PZA is also provided simultaneously. So far, the success of PZA in the TB treatment has been an extremely valuable finding; further investigation into the possible synergistic effects between PZA (or other TB drugs) and the {Au(PPh3)}+ moiety in vivo could be valuable toward identifying new drugs, especially for future treatment of emerging drug-resistant strains. Such studies are in progress in this laboratory.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c00071. Crystallographic Data (CIF)

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