Inhibition of Lysosomal Cysteine Proteases by a Series of Au(I) Complexes: A Detailed Mechanistic Investigation

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Complexes of gold(I) have long been used to treat rheumatoid arthritis although the precise biological targets of gold are not well understood. One intriguing therapeutic target of Au(I) is the cathepsin family of lysosomal cysteine proteases. Here, we present the inhibition of cathepsin B by a known Au(I)-based drug and a series of derivatives. The complexes investigated were reversible, competitive inhibitors with IC_{50} values ranging from 0.3 to 250 μM, depending on the substituents around the Au(I).

Introduction

It has long been recognized that Au(I) complexes can provide sustained relief from rheumatoid arthritis and other autoimmune disorders, but surprisingly little progress has been made in chrysotherapy over the years.[1] Sodium aurothiomalate (myochrysin) and sodium aurothioglucose (solganol), two of the compounds first reported to have antiarthritic activity, are still in use in the clinic today.[2,3] In the 1970s, an orally available compound, triethylphosphine(2,3,4,6-tetra-*O*-acetyl-β-1-D-thiopyranosato-S)gold(I) (auranofin, Figure 1), was introduced.[4,5]

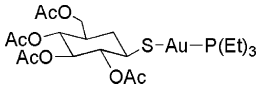

Figure 1. Molecular structure of auranofin.

Although all of these Au(I) compounds can dramatically affect the progress of rheumatoid arthritis, the molecular basis for the therapeutic effects is not well understood.

Gold(I) is a soft d^{10} metal ion that exhibits a preference for thiolate and phosphine donor ligands in a linear, two-coordinate geometry.[3] Au(I) complexes undergo facile ligand exchange in aqueous solutions, with the rate of ligand exchange increasing in the order $R_3P < RS^- < X^-$.[3,6] The lability of the ligands contributes to both the therapeutic activity of Au(I) antiarthritic compounds and the side effects observed with these drugs. In a biological setting, Au(I) interacts with cysteine and other thiol groups, especially those with low pK_a values.[7,8] Biomolecule−Au adducts, formed upon injection or ingestion of the drug by facile ligand replacement reactions, are believed to mediate the therapeutic effects of the metal ion.[3] Many pathways have been proposed for Au(I) antiarthritic activity, and it is likely that the therapeutic benefit is derived from a combination of biological effects. Gold may act at the cellular level by inhibiting T-cell proliferation and modulating the immune system.[9] Gold has also been shown to induce mitochondrial membrane permeability[10] and to inhibit osteoclast bone resorption.[11] At the level of transcription, gold may inhibit the activation of NF-κB, a transcription factor responsible for the production of TNF[a] and other key inflammatory cytokines.[12,13] Gold may also interfere with homeostasis of the copper(I) ion, binding to Cu(I)-responsive transcription factors and other Cu(I) trafficking proteins.[14]

The ability of Au(I) to undergo facile ligand exchange[3] indicates that gold may inhibit the activity of the cathepsins, a family of highly homologous, cysteine-dependent enzymes implicated in inflammation and joint destruction.[15,16] The cathepsins are intriguing biological targets of Au(I) for several reasons. First, gold is known to accumulate in the lysosomes, forming metal-rich cellular organelles that have been termed "aurosomes".[17] The lysosome is filled with degradative enzymes, many of which are cysteine-dependent, including the cathepsins. Second, gold is very thiophilic, with a high affinity for thiolates with low pK_a values, such as the activated cysteine residues found in the active sites of the cathepsins.[3,17] Third, the cathepsins are found in the extracellular environment in the inflamed joints of rheumatoid arthritis patients and participate in the joint destruction that is a hallmark of rheumatoid arthritis.[15,16,18] Finally, the cathepsins are involved in antigen processing and presentation and have been implicated in autoimmune disorders.[19] It is reasonable to consider that Au(I) exerts its anti-inflammatory and joint-protective effects at least in part by inhibiting the cathepsins. Gold(I)-mediated inhibition of the cathepsins has been reported in studies largely employing cellular lysates and impure enzyme preparations, resulting in conflicting reports of potency, reversibility, and mode of inhibition.[20−25] However, preliminary studies from our laboratory and others indicate that Au(I) does inhibit the cathepsins in vitro with moderate potency at levels that are likely to be therapeutically relevant.[22,25] In this manuscript, we report a detailed kinetic characterization of the interactions of auranofin and analogous Au(I) complexes with cathepsin B.

Experimental Section

General Considerations. All reagents and solvents were purchased from commercial sources and were used without further purification. Auranofin was purchased from MP Biomedicals, Inc. Human liver cathepsin B was purchased from Calbiochem. A standard fluorogenic cathepsin B substrate, Z-Phe-Arg-AMC, was purchased from Bachem California, Inc.[26] All enzyme assays were carried out using a buffer containing 20 mM sodium acetate, pH 5.5, 50 mM sodium chloride, 1.0 mM EDTA, 500 μM DTT, and 0.01% Brij 35. NMR spectra were recorded on a Bruker AC 250 MHz or a Bruker AM 360 MHz spectrometer. The samples for NMR were prepared in deuterated chloroform ($CDCl_3$), and the ^1H and ^{13}C NMR chemical shifts are reported in ppm relative to an internal standard of tetramethylsilane (TMS) or internal $CHCl_3$. Coupling constants (J) are reported in hertz (Hz) where relevant. ^{31}P NMR chemical shifts are reported relative to an external standard of 85% H_3PO_4. Chromatography was performed using

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[a] Abbreviations: TNF, tumor necrosis factor; AMC, 7-amino-4-methylcoumarin; DTT, dithiothreitol; SAR, structure−activity relationship.

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EMD silica gel (60−200 mesh). Elemental analyses were performed by Desert Analytics, Tucson, AZ (for elemental analysis results, see Table S2 in Supporting Information). Fluorescence data were collected (excitation at 360 nm and emission at 425 nm) using an Applied Biosystems Analyst AD fluorescence spectrophotometer.

Chemistry. General Synthesis of R′S−Au−PR$_3$.

(a) Synthesis of PR$_3$−Au−Cl. A solution of 2,2′-thiodiethanol (0.66 mmol) in 3.00 mL of acetone was added dropwise to a solution of sodium tetrachloroaurate(III) dihydrate (0.33 mmol) in 2.00 mL of deionized water at 0 °C. An almost immediate change of color of the mixture from yellow to colorless was observed. After the mixture was stirred for 10 min at 0 °C, the phosphine ligand (PR_3, 0.33 mmol) was dissolved in a minimum amount of acetone and added dropwise to the gold solution. A white precipitate formed almost immediately. This mixture was allowed to stir for 1 h at 0 °C followed by removal of the solvent on a rotary evaporator. The resulting white solid was washed with a small amount of cold acetone/deionized water (1:2) solution and subjected to an extractive workup using CH_2Cl_2/H_2O. The chloro(trialkylphosphine)gold complex (PR_3−Au−Cl) obtained after the removal of CH_2Cl_2 could be dried and characterized if desired but was pure enough to use directly in the next step.

(b) Synthesis of R′S−Au−PR$_3$. The purified chloro(trialkylphosphine)gold complex (PR_3−Au−Cl) was dissolved in a minimum amount of acetone with stirring for 5 min at 0 °C. The thiol ligand (R′SH, 0.33 mmol) was dissolved in 1.0 mL of acetone, deprotonated using 1.0 equiv of NaOH, and added dropwise to this mixture. The mixture was stirred for 3 h. The solid obtained after the removal of acetone was extracted with CH_2Cl_2/deionized water. The CH_2Cl_2 was removed under vacuum, and the complex obtained was characterized using NMR and elemental analysis. All of the compounds (**1**−**5**) were synthesized using this general method.

Synthesis of (4-Nitrobenzenethiolate)(tris(4-methoxyphenyl)-phosphine)gold(I) Complex ((*p*-C$_6$H$_4$NO$_2$)SAuP(*p*-C$_6$H$_4$OCH$_3$)$_3$, 1). Complex **1** was recrystallized through slow evaporation of an ethanol/CH_2Cl_2 solution, yielding clear-yellow rectangular plates (52% crystalline yield). In addition to characterization by NMR and elemental analysis, this complex was also characterized using X-ray crystallography. Pertinent crystallographic information about the complex, bond distances and angles, atomic coordinates, and equivalent isotropic displacement parameters are provided in the Supplementary Information. ^{1}H NMR (250 MHz, $CDCl_3$): δ 3.858 (9H, s), 6.988 (6H, dd, J = 1.75, 8.75), 7.449 (6H, dd, J = 9, 12.25), 7.672 (2H, d, J = 8.75), 7.930 (2H, d. J = 8.75). ^{13}C NMR (90 MHz, $CDCl_3$): δ 55.462, 114.818, 114.965, 123.077, 132.055, 135.443, 135.608. ^{31}P NMR (145 MHz, 85% H_3PO_4): δ 35.436.

Synthesis of (2,3,4,6-Tetra-*o*-acetyl-1-thio-β-D-glucopyranosato-s)(triphenylphosphine)gold(I) Complex (C$_{14}$H$_{19}$O$_9$SAuP(C$_6$H$_5$)$_3$, 2). The complex was obtained as a white solid (79% overall yield). ^{1}H NMR (360 MHz, $CDCl_3$): δ 1.892 (3H, s), 1.977 (3H, s), 2.017 (3H, s), 2.050 (3H, s), 3.760 (1H, m), 4.114 (1H, dd, J = 2.16, 12.24), 4.218 (1H, dd, J = 4.86, 12.42), 5.139 (4H, m), 7.522 (15H, m). ^{13}C NMR (90 MHz, $CDCl_3$): δ 20.638, 20.704, 21.106, 62.822, 68.908, 74.182, 75.644, 77.554, 82.862, 129.042, 129.081, 129.228, 131.638, 134.141, 134.307, 170.345. ^{31}P NMR (145 MHz, 85% H_3PO_4): δ 39.364.

Synthesis of (2,3,4,6-Tetra-*o*-acetyl-1-thio-β-D-glucopyranosato-s)(tris(2-methoxyphenyl)phosphine)gold(I) Complex (C$_{14}$H$_{19}$O$_9$SAuP(*o*-C$_6$H$_4$OCH$_3$)$_3$, 3). The complex was obtained as a white solid (40% overall yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.866 (3H, s), 1.914 (3H, s), 1.969 (3H, s), 2.008 (3H, s), 3.715 (9H, s), 3.889 (1H, m), 4.134 (2H, m), 5.070 (4H, m), 6.959 (6H, m), 7.174 (3H, t), 7.486 (3H, t). ^{13}C NMR (90 MHz, $CDCl_3$): δ 20.661, 20.709, 20.836, 55.788, 62.740, 68.835, 74.478, 75.612, 82.703, 111.565, 111.616, 116.388, 117.061, 120.723, 120.845, 132.816, 134.906, 135.002, 161.227, 161.284, 169.530, 170.416, 170.913. ^{31}P NMR (145 MHz, 85% H_3PO_4): δ 32.403.

Synthesis of (Benzene thiolate)(triphenylphosphine)gold(I) Complex (C$_6$H$_5$SAuP(C$_6$H$_5$)$_3$, 4). The final product was purified using column chromatography ($CHCl_3$/acetone (5:1)), which yielded the complex as a white solid (73% overall yield). ^{1}H NMR (360 MHz, $CDCl_3$): δ 6.987 (1H, m), 7.101 (1H, m), 7.281 (3H, m), 7.529 (15H, m). ^{13}C NMR (90 MHz, $CDCl_3$): δ 128.428, 128.568, 129.116, 129.262, 131.945, 132.032, 132.111, 134.111, 134.268. ^{31}P NMR (145 MHz, 85% H_3PO_4): δ 39.033.

Synthesis of Chloro(triphenylphosphine)gold(I) Complex (ClAuP(C$_6$H$_5$)$_3$, 5). The desired complex was obtained as a white solid (82% overall yield). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.496 (15H, m). ^{13}C NMR (90 MHz, $CDCl_3$): δ 129.331, 129.454, 132.168, 134.222, 134.368. ^{31}P NMR (145 MHz, 85% H_3PO_4): δ 33.802.

Collection and Reduction of X-ray Data. X-ray diffraction data were collected on a Bruker SMART APEX CCD diffractometer with graphite monochromated Mo Kα radiation (λ = 0.710 73 Å) at low temperature (128 K). The unit cell parameters for the compound were obtained from the least-squares refinement of reflections from 60 collected frames using the SMART[27] software package. A hemisphere of data was collected up to a resolution of 0.75 Å, and the intensity data were processed using the Saint Plus program.[28] The structure was solved by using the direct methods program XS, part of the SHELXTL[29] program package, and refined by least-squares methods using SHELX with 5579 independent reflections within the range θ = 1.93−27.47° (completeness 93.7%). Empirical absorption corrections were calculated and applied by using the program SADABS.[30] The positions of the hydrogen atoms were calculated and refined in a riding manner along with the attached carbons.

Enzyme Activity and Inhibition Assays. Standard conditions used to determine the activity of the enzyme against the fluorogenic substrate *Z*-Phe-Arg-AMC (stock solution prepared in DMSO) were described previously.[25] The inhibition assays were performed using a fixed enzyme concentration of 2.02 nM and a fixed substrate concentration of 12.5 μM. Prior to the reaction, the enzyme was incubated for 30 min with 500 μM dithiothreitol (DTT). Stock solutions and serial dilutions of the synthesized linear gold complexes were made in DMSO. Aliquots of substrate and inhibitor were taken such that the final concentration of DMSO in each assay was held constant, never exceeding 5% of the total reaction volume. Enzyme activity was measured in the presence of between 50 nM and 300 μM Au(I) complex at room temperature (21−24 °C) in 96-well plates. Assays were conducted in triplicate at each concentration. The increase in fluorescence as the substrate was hydrolyzed was measured every 60 s for 30 min (λ_{ex} = 360 nm, λ_{em} = 425 nm). Relative fluorescence units were converted into concentration of 7-amino-4-methylcoumarin (AMC) produced using a standard curve of AMC fluorescence.

Reversibility Assays. The reversibility of Au(I)-mediated cathepsin B inhibtion was demonstrated in experiments in which 2.00 nM enzyme was preincubated with 500 μM DTT for 30 min, followed by incubation of the activated enzyme with either 200 μM auranofin or 250 nM complex **2** for 30 min. The incubated cathepsin B−Au(I) mixture and a control reaction with no Au(I) present were subjected to an additional 30 min of incubation with between 0 and 12.5 mM L-cysteine. The substrate concentration was fixed at 12.5 μM, and the activity of cathepsin B was measured in triplicate at each L-cysteine concentration.

Lineweaver−Burk Plot Assays. The activity of cathepsin B was measured in the absence and presence of different fixed concentrations of inhibitor at a series of substrate concentrations. Assays were conducted in triplicate for each concentration of substrate. Inhibitor concentrations were chosen such that they were approximately 10-fold higher than, 10-fold lower than, and close to the IC_{50} value of the complex investigated. The reciprocal of the reaction rate was plotted as a function of the reciprocal of the substrate concentration for each concentration of inhibitor.

Results and Discussion

Chemistry. Auranofin analogues were synthesized and isolated in pure form by using a modification of a procedure in the literature (Scheme 1).[4] In all cases, the material obtained was analytically pure (see Table S1) and the yields reported in

Scheme 1. Synthesis of Au(I) Complexes[5,a]

$$Na^+AuCl_4^- \xrightarrow[(ii)]{(i)} Cl\text{-}Au\text{-}PR_3 \xrightarrow{(iii)} R'S\text{-}Au\text{-}PR_3$$

[a] Conditions: (i) 2,2′-thiodiethanol, 0 °C, 10 min; (ii) PR_3, 0 °C, 1 h; (iii) R′SH, 1 M NaOH, 0 °C, 3 h.

the Experimental Section are overall yields based on the amount of gold used in each reaction. Any unreacted gold can be recycled.[31] In addition to a full spectroscopic and analytical characterization of each compound, we were able to obtain high-quality single crystals of complex **1** and determine an X-ray crystal structure of this compound. An ORTEP diagram showing 50% probability ellipsoids is presented in Figure 2. Although

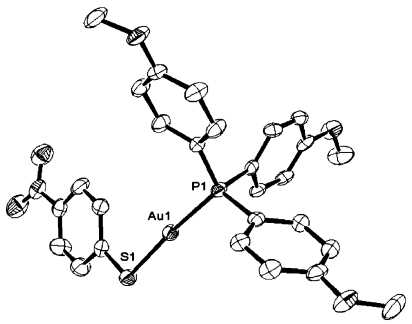

Figure 2. ORTEP diagram of complex **1**, showing 50% probability ellipsoids. The hydrogen atoms have been omitted for clarity.

the thiolate and phosphine ligands are considerably different in auranofin and compound **1**, the coordination environment around the Au(I) ion remains essentially the same.[32] Both compounds exhibit a linear, two coordinate geometry with a S−Au−P bond angle of 175.13° ± 6° in complex **1** and 173.6° ± 1° in auranofin. The Au−P distance is essentially identical (2.259 ± 3 Å in auranofin and 2.258 ± 2 Å in **1**), and the Au−S distance is only slightly longer (2.299 ± 2 Å) in complex **1** compared to the distance in auranofin (2.293 ± 3 Å). It appears that changing the identity of the thiolate and phosphine ligands has little effect on the ligand geometry around the Au(I) ion. This conclusion is supported by other structurally characterized phosphine−Au(I)−thiolate compounds.[33]

Mechanism of Auranofin-Mediated Inhibition of Cathepsin B. The cathepsins are thiol-dependent enzymes, requiring an activated cysteine thiolate in their active sites that serves as a nucleophile in the hydrolysis of peptide bonds.[15,16,34] Because of the thiophilicity of Au(I) and the ability of auranofin to undergo thiolate ligand exchange reactions, this active site cysteine residue is a likely target of Au(I)-based drugs. Previous work by our laboratory has demonstrated that auranofin inhibits cathepsin B with an IC_{50} value of approximately 250 μM.[25] In this study, we undertook an investigation of the mechanism of inhibition as well as the roles played by each of the ligands in cathepsin inhibition.

To investigate the reversibility of inhibition, the activated enzyme was incubated with 200 μM auranofin for 30 min followed by addition of increasing concentrations of L-cysteine and a fixed concentration of substrate. The activity of the enzyme−gold−L-cysteine mixture was compared to the activity of the enzyme under the same conditions in the absence of gold. The cathepsin B activity was inhibited by Au(I) in the absence of L-cysteine but slowly recovered with the increase in L-cysteine concentration. The final steady-state enzyme activity was equal to that of the control, demonstrating that auranofin inhibition of cathepsin B can be reversed by the addition of excess of L-cysteine (Figure 3). These data are in line with the fact that

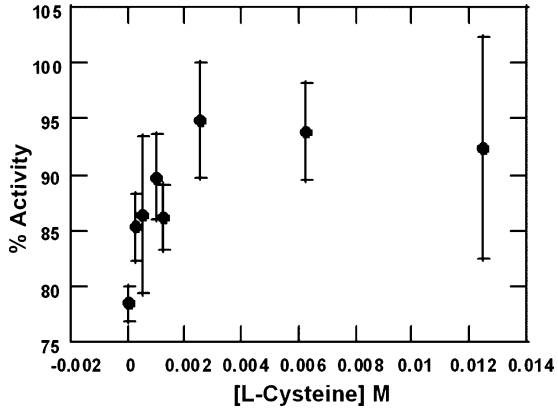

Figure 3. Reversibility of auranofin-mediated cathepsin B inhibition by addition of excess cysteine.

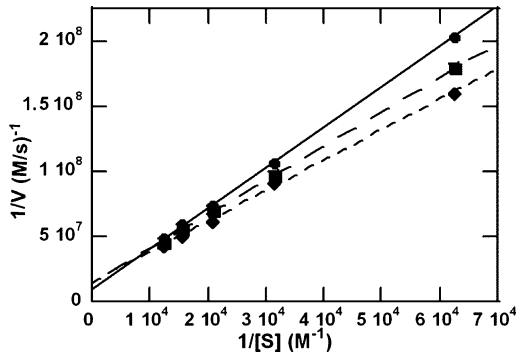

Figure 4. Lineweaver−Burk plot in the absence (◆) and in the presence of 100 μM (■) and 250 μM (●) auranofin.

Au(I) complexes are kinetically labile and undergo facile thiolate ligand exchange reactions. L-Cysteine competes with the enzyme for coordination of Au(I). Furthermore, in the range of concentrations studied, L-cysteine itself has no effect on the activity of cathepsin B in the absence of Au(I).

If auranofin binds in the active site of cathepsin B, competitive inhibition kinetics would be expected. The mode of auranofin-mediated cathepsin B inhibition was investigated using a series of substrate and inhibitor concentrations. The double-reciprocal plot in Figure 4 is consistent with a competitive inhibition scheme in which Au(I) competes with substrate for binding in the active site of the enzyme. Taken together, these data indicate that auranofin is a moderately potent, competitive, reversible inhibitor of cathepsin B.

Effect of Phosphine Ligand on Inhibition. There is considerable evidence in the literature that on the time scale of our reactions, the phosphine ligand remains coordinated to the Au(I) in vitro.[35,36] Furthermore, investigations into the pharmacokinetics and pharmacodynamics of auranofin and related compounds provide evidence that the phosphine ligand plays a role in the therapeutic utility of the drug.[4,37,38] To investigate the effect that changing the phosphine ligand has on cathepsin inhibition, we synthesized the series of compounds shown in Table 1. In these compounds, the phosphine ligands add varying degrees of steric bulk to the Au(I)−phosphine fragment, the portion of the molecule that presumably interacts with the active site of cathepsin B. One measure of the steric bulk of a phosphine−metal complex is the cone angle (Table 1).[39,40] From the perspective of the enzyme, a cone angle of greater than 180° would indicate that the substituents on the phosphine ligand are directed toward the active site. A complex with a very large cone angle would be expected to experience unfavorable steric interactions with the residues in the active site of the enzyme,

Table 1. Effect of Changing the Phosphine Ligand of R′S−Au−PR_3 Complexes[a]

R	cone angle, deg	electronic parameter, cm^{-1}	IC_{50}, μM
CH_2CH_3	132	2061.7	~250
C_6H_5 (**2**)	145	2068.9	0.339
o-$C_6H_4OCH_3$ (**3**)	194	2066.6	>250

[a] For all the complexes, R′S = $C_{14}H_{19}O_9S$.

whereas compounds with a small cone angle may not interact well with the active site. As outlined in Table 1, the IC_{50} values of the gold compounds vary dramatically in response to changes in the phosphine ligand. The least potent inhibitor has an IC_{50} value over 700 times higher than that of the most potent inhibitor.

Cathepsin B has a spacious active site and selectively binds peptide substrates with aromatic side chains, particularly those containing a phenylalanine residue one amino acid removed from the scissile bond.[41,42] It is therefore not surprising that Au(I) complexes with larger hydrophobic phosphine ligands interact more favorably in the active site of cathepsin B. However, it appears that compounds such as **3**, with very large cone angles (therefore, a large amount of steric bulk directed along the P−Au bond toward the enzyme), are too bulky to allow a favorable Au(I)−enzyme interaction. Auranofin, with a small cone angle, also has a very high IC_{50} value, indicating that it does not interact favorably with the active site residues. In addition to the steric bulk of the phosphine ligand, the electronic properties of the ligand may also contribute to the observed differences in inhibitory potency of the complexes. A detailed study to address this question is underway in our laboratory. However, the current results are intriguing because they imply that more extensive SAR studies could identify Au(I)-based cathepsin inhibitors with significantly enhanced potency. Molecular modeling may also aid in the design of more potent, selective Au(I)-based cathepsin inhibitors with therapeutic potential.

Effect of Thiolate Ligand on Inhibition. To investigate the effect that different thiolate ligands have on the inhibition of cathepsin B, we synthesized a series of compounds shown in Table 2. On the basis of the known lability of Au(I) thiolates,

Table 2. Effect of Changing the Thiolate Ligand of R′S−Au−PR_3 Complexes[a]

R′S	IC_{50}, μM
$C_{14}H_{19}O_9S$ (**2**)	0.339
C_6H_5S (**4**)	0.928
Cl (**5**)	0.334

[a] For all the complexes, PR_3 = $P(C_6H_5)_3$.

we anticipate that the thiolate ligand is the leaving group in this reaction. Alternatively, a halide ion could serve as the leaving group, as is the case for complex **5**. If the thiolate or halide ligand undergoes facile exchange with a cathepsin B derived moiety, the size and identity of this ligand should not affect the inhibitory potency of the complex, in principle. Compounds **2**, **4**, and **5**, which all contain triphenylphosphine and a different thiolate or halide ligand, inhibit cathepsin B with very similar IC_{50} values, indicating that the identity of the leaving group in this reaction has little effect on inhibition.

Changing the Phosphine and Thiolate Ligands Does Not Change the Mechanism of Inhibition. By variation of the phosphine and thiolate ligands of auranofin, a cathepsin B inhibitor with 700-fold greater potency, compound **2**, has been identified. To ensure that the mechanism of inhibition was not altered, we carried out studies of the reversibility and mode of

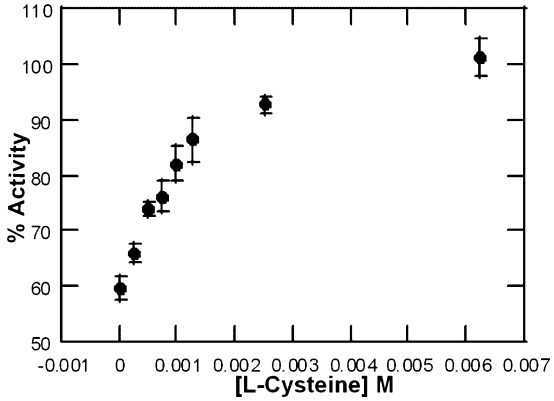

Figure 5. Reversibility of complex **2** mediated cathepsin B inhibition by addition of excess cysteine.

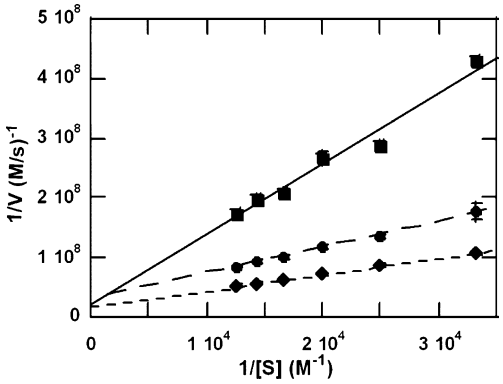

Figure 6. Lineweaver−Burk plot in the absence (◆) and in the presence of 2.5 μM (■) and 250 nM (●) complex **2**.

cathepsin B inhibition by **2**. As shown in Figure 5, the inhibition of cathepsin B by compound **2** can be reversed by the addition of excess L-cysteine. Furthermore, as illustrated in Figure 6, **2** appears to be a competitive inhibitor of cathepsin B. These results further highlight the potential for future inhibitor development based on rational design of the Au(I) complex.

Conclusions

Linear Au(I) complexes have been synthesized and characterized, each of which inhibits cathepsin B activity in vitro. On the basis of the thiophilicity and relative lability of the Au(I) complexes investigated, this inhibition is expected to be competitive and reversible. The data reported here are in agreement with this prediction. We propose that Au(I) binds in the active site of cathepsin B, likely inhibiting enzyme activity by interacting directly with the active site cysteine residue. Of the compounds investigated, complex **2** is the best inhibitor in the series with an IC_{50} value of 339 nM and is over 700 times more potent than auranofin. Although variations in the phosphine ligand can have dramatic effects on the ability of the complexes to inhibit cathepsin B, changing the thiolate ligand has little effect on the inhibition. This information should prove to be valuable in the design of even more potent gold-based inhibitors in the future. The active site of cathepsin B is surrounded by a series of substrate recognition pockets that can be exploited in inhibitor design. By incorporation of substituents that will interact favorably with the enzyme active site into the phosphine ligand of the Au(I) complex, it may be possible to design inhibitors with enhanced potency.

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Supporting Information Available: Crystallographic data in CIF format, Table S1 summarizing the X-ray crystallographic data for compound **1**, Table S2 containing the elemental analysis data for compounds **1**−**5**, and Figures S1−S7 showing IC_{50} curves for complexes **1**−**5** and comparing IC_{50} curves for complexes with varying phosphine and thiolate ligands. This material is available free of charge via the Internet at http://pubs.acs.org.

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