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A Synthetic Model for the Inhibition of Glutathione Peroxidase by Antiarthritic Gold Compounds

Krishna P. Bhabak and Govindasamy Mugesh*

Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore 560012, India

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In this paper, inhibition of the glutathione peroxidase activity of two synthetic organoselenium compounds, bis-[2-(*N*,*N*-dimethylamino)benzyl]diselenide (**5**) and bis[2-(*N*,*N*-dimethylamino)benzyl]selenide (**9**), by gold(I) thioglucose (**1**), chloro(triethylphosphine)gold(I), chloro(trimethylphosphine)gold(I), and chloro(triphenylphosphine)gold(I) is described. The inhibition is found to be competitive with respect to a peroxide (H_2O_2) substrate and noncompetitive with respect to a thiol (PhSH) cosubstrate. The diselenide **5** reacts with PhSH to produce the corresponding selenol (**6**), which upon treatment with 1 equiv of gold(I) chlorides produces the corresponding gold selenolate complexes **11**−**13**. However, the addition of 1 equiv of selenol **6** to complexes **11**−**13** leads to the formation of bis-selenolate complex **14** by ligand displacement reactions involving the elimination of phosphine ligands. The phosphine ligands eliminated from these reactions are further converted to the corresponding phosphine oxides ($R_3P{=}O$) and selenides ($R_3P{=}Se$). In addition to the replacement of the phosphine ligand by selenol **6**, an interchange between two different phosphine ligands is also observed. For example, the reaction of complex **11** having a trimethylphosphine ligand with triphenylphosphine produces complex **13** by phosphine interchange reactions via the formation of intermediates **15** and **16**. The reactivity of selenol **6** toward gold(I) phosphines is found to be similar to that of selenocysteine.

Introduction

Gold compounds such as gold thiomalate (myochrisine, GTM), gold thioglucose (**1**, solganol, GTG), and auranofin (**2**, AUR) have been employed as therapeutic agents for rheumatoid arthritis (RA) for many years (Figure 1).[1] On the basis of various effects of gold compounds in a number of different model systems, several pathways have been proposed to explain the mechanisms of action of gold compounds in RA,[2] but none has been generally accepted as the mechanism by which gold compounds alter the course of RA. Recent studies show that the gold drugs GTG and AUR effectively inhibit certain selenoenzymes such as glutathione peroxidase (GPx),[3] type I iodothyronine deiodinase (ID-1),[4] and thioredoxin reductase (TrxR),[5] indicating that gold compounds may exert some of their pharmacological effects by inhibiting one or more selenoenzymes.

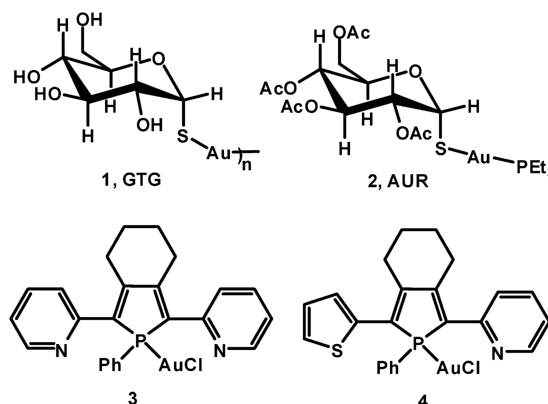

Figure 1. Chemical structures of some therapeutic gold(I) compounds.

* To whom correspondence should be addressed. E-mail: mugesh@ipc.iisc.ernet.in.

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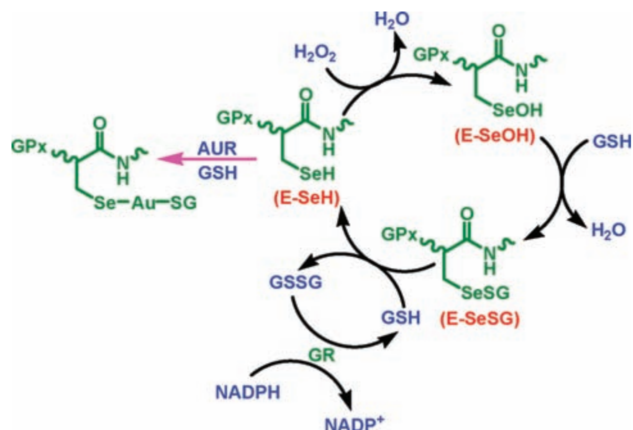

Figure 2. Proposed GPx catalytic mechanism and inhibition by AUR.

GPx is an antioxidant selenoenzyme that protects various organisms from oxidative damage by catalyzing the reduction of hydrogen peroxide and other organic peroxides with the help of glutathione (GSH) as the reducing agent.[6] The active site of GPx includes a selenocysteine residue, which undergoes a series of oxidation and reduction reactions during the enzyme catalytic cycle (Figure 2). The reaction of the selenol moiety (E-SeH) at the active site of GPx with H_2O_2 produces the selenenic acid (E-SeOH) intermediate, which upon reaction with GSH produces the corresponding selenenyl sulfide (E-Se-SG). The attack of a second 1 equiv of GSH at the −Se−S− linkage eliminates glutathione disulfide (GSSG) and regenerates E-SeH.[6c,7] In both in vivo and in vitro systems, GSSG produced during the catalytic cycle is reduced back to GSH by the NADPH-dependent glutathione reductase (GR) (Figure 2). Therefore, the GPx activity is generally measured indirectly by following the GR-catalyzed reduction of GSSG.[7]

It has been reported that the gold drugs GTG (**1**) and AUR (**2**) inhibit the GPx activity probably by reacting with E-SeH of the enzyme to form a stable gold selenolate complex.[3] Furthermore, it has been postulated that gold compounds must undergo ligand displacement reactions with GSH to produce the gold−GSH complex $[Au(SG)_2]^-$, which reacts with E-SeH to produce the corresponding E−Se−Au−SG complex (Figure 2).[3c] Similar to measurement of the GPx catalytic activity, the inhibition of GPx by gold compounds is routinely followed by a GR−GSSG coupled assay.[3a,c] However, recent evidence suggests that the gold compounds such as **3** and **4** that inhibit selenoenzymes can also inhibit GR by reacting with the active-site cysteine residues.[8] Therefore, the indirect measurement of the GPx activity by using the GR−GSSG coupled assay may lead to a complication in the mechanism of the inhibition of GPx by gold compounds.

To understand the effect of gold compounds on the GPx activity and to avoid the complication in the GR−GSSG coupled assay, we have used bis[2-(*N*,*N*-dimethylamino)benzyl]diselenide (**5**), which has been shown to mimic GPx by reducing H_2O_2 in the presence of an aromatic thiol such as benzenethiol (PhSH).[9] By using PhSH instead of GSH, the GPx activity can be directly measured by following the formation of diphenyl disulfide (PhSSPh).[10] The reduction of the −Se−Se− bond in compound **5** by thiols leads to the formation of the corresponding selenol (**6**), which is responsible for the GPx-like catalytic activity of **5**.[9] The formation of selenol **6** was further confirmed from the reaction of selenol **6** with iodoacetic acid, which produces the corresponding monoselenide as the only product (Figures S22 and 23 in the Supporting Information). If the inhibition of native GPx by gold(I) compounds occurs through the formation of a gold selenolate complex as shown in Figure 2, the catalytic activity of GPx mimic **5** should also be inhibited by gold(I) complexes. In this paper, we describe inhibition of the GPx-like antioxidant activity of the diselenide **5** and the corresponding monoselenide **9** by GTG and related gold(I) compounds. We also describe that inhibition of the GPx activity by gold(I) compounds is due to the interaction between the selenol moiety and gold(I) compounds to form gold selenolate complexes.

Experimental Section

General Procedure. Compounds **5**[11a] and **9**[11b] were synthesized by following literature methods. ^{1}H (400 MHz), ^{13}C (100.5 MHz), ^{31}P (161.9 MHz), and ^{77}Se (76.3 MHz) NMR spectra were obtained on a Bruker 400 MHz NMR spectrometer. Chemical shifts are cited with respect to $SiMe_4$ (^{1}H and ^{13}C) as internal standards and H_3PO_4 (^{31}P) and Me_2Se (^{77}Se) as external standards. Mass spectral studies were carried out on a Bruker Daltonics Esquire 3000plus mass spectrometer with electrospray ionization mass spectrometry (ESI-MS) mode analysis.

Synthesis of 6. To a solution of compound **5** (20.0 mg, 0.047 mmol) in $CDCl_3$in an NMR tube was added dithiothreitol (DTT;

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11.0 mg, 0.070 mmol), and the solution was shaken well for uniform mixing. The formation of the selenol **6** was monitored by the disappearance of the peak for compound **5** (426 ppm) and the appearance of a new peak at 38 ppm in ^{77}Se NMR. Because of the rapid aerial oxidation of selenol **6** to the corresponding diselenide **5**, it could not be characterized by other techniques.

However, the treatment of iodoacetic acid (2 equiv) to selenol **6** produces corresponding monoselenide, which was further characterized by ^{77}Se NMR and mass spectrometric techniques. For the monoselenide compound: ^{77}Se NMR (ppm, $CDCl_3$): δ 246. ESI-MS. Calcd for $C_{11}H_{15}NO_2Se$ [$(M + H)^+$]: *m/z* 274.0346. Found: *m/z* 274.0341 (Figures S22 and S23 in the Supporting Information).

Synthesis of 10. To a solution of compound **9** (10 mg, 0.028 mmol) in a mixture of chloroform and methanol (4:1) was added H_2O_2 (4.0 μL, 0.034 mmol), and the resulting solution was stirred for 30 min at room temperature. The solvent was removed under vacuum to obtain **10** as a white amorphous solid in quantitative yield. ^1H NMR ($CDCl_3$, ppm): δ 2.07 (s, 12H), 3.36−3.40 (d, 2H, $J = 13.6$ Hz), 3.50−3.53 (d, 2H, $J = 13.6$ Hz), 7.16−7.17 (d, 2H, $J = 4.4$ Hz), 7.28−7.30 (t, 4H, $J = 4.4$ Hz), 7.72−7.73 (d, 2H, $J = 3.6$ Hz). ^{13}C NMR ($CDCl_3$, ppm): δ 43.6, 62.0, 127.5, 127.7, 127.8, 129.1, 137.9, 141.7. ^{77}Se NMR ($CDCl_3$, ppm): δ 857. ESI-MS. Calcd for $C_{18}H_{24}N_2OSe$ [$(M + H)^+$]: *m/z* 365.1132. Found: *m/z* 365.1125.

General Synthesis of Compounds 11−13. To the selenol **6**, generated from the corresponding diselenide **5**, was added a stoichiometric amount of R_3PAuCl (R = Me, Et, and Ph) in $CDCl_3$ in an NMR tube. The yellow solution of selenol turned almost colorless immediately upon the addition of gold(I) chlorides. The formation of phosphinegold(I) selenolate species (**11**−**13**) was characterized by ^{31}P and ^{77}Se NMR spectroscopy and mass spectrometric techniques. Compound **11**. ^{31}P NMR ($CDCl_3$, ppm): δ −0.62. ^{77}Se NMR ($CDCl_3$, ppm): δ 87. ESI-MS. Calcd [$(M + H)^+$]: *m/z* 488.0321. Found: *m/z* 487.8654. Compound **12**. ^{31}P NMR ($CDCl_3$, ppm): δ 38.31. ^{77}Se NMR ($CDCl_3$, ppm): δ 76. ESI-MS. Calcd [$(M + H)^+$]: *m/z* 530.0790. Found: *m/z* 529.9380. Compound **13**. ^{31}P NMR ($CDCl_3$, ppm): δ 37.50. ^{77}Se NMR ($CDCl_3$, ppm): δ 74. ESI-MS. Calcd [$(M + H)^+$]: *m/z* 674.0790. Found: *m/z* 673.9761.

HPLC Assay. In this assay, we employed a mixture containing a 1:2 molar ratio of PhSH and peroxide in methanol at room temperature as our model system. Runs with and without catalyst were carried out under the same conditions. In inhibition experiments, increasing concentrations of the inhibitor were added to the reaction mixture along with the catalyst. Periodically, aliquots were injected into the reverse-phase C_{18} (Atlantis, 4.6 × 250 mm, 5 μm) column and eluted with methanol and water (9:1), and the concentrations of the product diphenyl disulfide (PhSSPh) were determined at 254 nm using pure PhSSPh as an external standard. The amount of disulfide formed during the course of the reaction was calculated from the calibration plot for the standard (PhSSPh).

Inhibition of the GPx Activity by Gold Drugs. The gold(I) compounds, such as GTG, and R_3PAuCl (R = Me, Et, and Ph) were used as inhibitors of GPx mimics. Inhibition experiments of the GPx-like activities of test compounds by gold(I) drugs were performed in the presence of an aromatic thiol (PhSH) and different hydroperoxides [hydrogen peroxide (H_2O_2), cumene hydroperoxide (Cum-OOH), and *tert*-butyl hydroperoxide (*t*-BuOOH)] using the reverse-phase HPLC method. Each reaction rate for the reduction of peroxide was measured at least three times and was corrected for the background reaction between peroxide and thiol. The rate for the reduction of peroxides by selenium compounds in the absence of gold(I) compounds (inhibitors) was considered as 100% (control activity), and inhibition of the GPx activity in the presence of various concentrations of gold(I) compounds (inhibitors) was expressed as a percentage of the control activity. The IC_{50} values represent the concentrations of gold(I) compounds required to inhibit 50% of the catalytic activity. These values were calculated by plotting the percent control activity with respect to the concentration of gold(I) compounds. The data points were plotted as a sigmoidal curve fitting using *Origin 6.1* software.

Results and Discussion

Mechanism of Inhibition of the GPx Activity of 5 by Gold(I) Compounds. To understand the mechanism by which GTG and other gold(I) compounds inhibit the GPx activity, we have studied the effect of GTG, Et_3PAuCl, Me_3PAuCl, and Ph_3PAuCl on the reduction of peroxides by compound **5** in the presence of PhSH. The advantage of using PhSH as the thiol cofactor is that the activity of **5** can be followed directly by measuring the rate of formation of PhSSPh.[10] Because there is no GR or NADPH involved, inhibition of the GPx activity by gold(I) compounds must be due to the reaction between selenol (**6**) and gold(I) compounds. The inhibition experiments were carried out by using H_2O_2, *t*-BuOOH, and Cum-OOH as substrates and PhSH as a cosubstrate. Interestingly, the trialkyl- or triphenylphosphinegold compounds inhibited the GPx activity much stronger than GTG, supporting the assumption that the higher inhibitory effect of AUR as compared with GTG is due to the presence of a triethylphosphine moiety in AUR, which may stabilize the Au−Se bond (Table 1). In general, the gold(I) chlorides exhibited higher activity (lower IC_{50} values) as compared to GTG in all three peroxide assays (H_2O_2, *t*-BuOOH, and Cum-OOH) employed in the present study, indicating that gold(I) chlorides are better than GTG as inhibitors in the presence of PhSH.

Furthermore, inhibition of the GPx activity of compound **6** is due to a direct interaction between the selenol moiety and gold(I) complexes because the thiol cosubstrate (PhSH) present in the assay does not react with the gold(I) complexes (Figures S17−S19 in the Supporting Information). Therefore, inhibition of the GPx activity by gold(I) complexes is expected to be competitive with respect to peroxide. On the basis of the inhibition of GPx by AUR (Figure 2), the selenol **6** can react either with the peroxide (H_2O_2) to produce the corresponding selenenic acid **7** or with GTG to produce the corresponding gold selenolate complex **8** (Figure 3). To understand the nature of inhibition of the GPx activity, we have performed detailed kinetic experiments at different concentrations of GTG by varying the concentrations of the

Table 1. Comparison of IC_{50} Values for Inhibition of the GPx Activity of **5** by Some Gold(I) Compounds

gold(I) compounds	IC_{50} values (μM)[a]		
	H_2O_2	*t*-BuOOH	Cum-OOH
GTG	46.9 ± 1.8	40.6 ± 0.3	43.2 ± 0.5
Et_3PAuCl	16.8 ± 0.1	14.3 ± 0.5	14.7 ± 0.2
Me_3PAuCl	18.2 ± 1.0	17.3 ± 0.7	19.4 ± 0.3
Ph_3PAuCl	13.7 ± 0.3	13.7 ± 0.6	12.4 ± 0.5

[a] The concentration required to inhibit 50% of the catalytic activity. Assay conditions: inhibitor (variable), compound **5** (2.5 μM), PhSH (5 mM), and peroxide (10 mM) in MeOH at 23 °C.

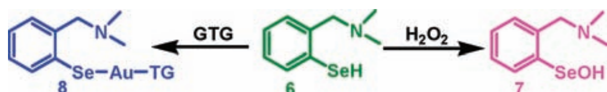

Figure 3. Two possible modes of reaction for the selenol **6** in the presence of H_2O_2 and GTG.

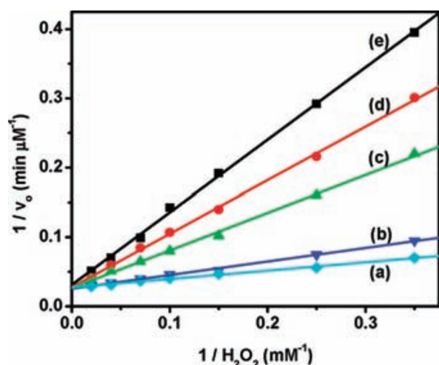

Figure 4. Lineweaver−Burk plots obtained for inhibition of the GPx activity catalyzed by **5** in the presence PhSH at various concentrations of GTG. [GTG]: (a) 50 μM; (b) 60 μM; (c) 70 μM; (d) 90 μM; (e) 110 μM. For each concentration of GTG, the substrate (H_2O_2) concentration was varied, while keeping the concentration of PhSH constant.

substrate (H_2O_2) and thiol cosubstrate for each GTG concentration. The initial rates (v_0) were calculated for each concentration of GTG and H_2O_2 at a fixed concentration of PhSH. The Lineweaver−Burk plots obtained by plotting $1/v_0$ versus $1/[H_2O_2]$ show linear lines for different concentrations of GTG, and all of these straight lines intersect the ordinate with almost identical V_{max} values (Figure 4), suggesting that inhibition of the GPx activity of **5** by GTG is competitive with respect to the substrate, H_2O_2.

Although inhibition of the GPx activity of **6** by GTG is competitive with respect to the peroxide substrates, the comparable IC_{50} values obtained for different peroxides (Table 1) indicate that the nature of peroxide has little effect on the inhibition. This is in agreement with our previous observations that the nature of peroxide does not affect the GPx-like activity of ebselen and related compounds.[10d]

We carried out further kinetic experiments with different concentrations of PhSH and GTG at a fixed concentration of H_2O_2 to find out the effect of the thiol concentration on the inhibition. In contrast to the effect of H_2O_2 on the inhibition, the Lineweaver−Burk plots obtained from these experiments show parallel lines, which do not intersect at a common point (Figure 5), indicating that inhibition of the GPx activity of **5** by GTG is noncompetitive with respect to the thiol cosubstrate. Although selenol **6** reacts with the inhibitor (GTG) to produce the corresponding gold selenolate complex (**8**), it does not directly react with the thiol (PhSH) present in the assay system. Therefore, any variation in the concentration of the thiol cosubstrate does not affect the reactivity of selenol **6** toward GTG (Figure 6). This is also reflected in the noncompetitive nature of the inhibition of compound **5** by GTG with respect to PhSH. Furthermore, GTG and other gold(I) compounds do not react with other catalytic intermediates such as selenenic acid or selenenyl sulfide in the redox cycle. However, the inactivity of the electrophilic gold compounds toward selenenic acid or selenenyl sulfide is not surprising because the selenium atoms in these intermediates are also electrophilic in nature and an attack of gold at selenium in these intermediates is not a favored process.

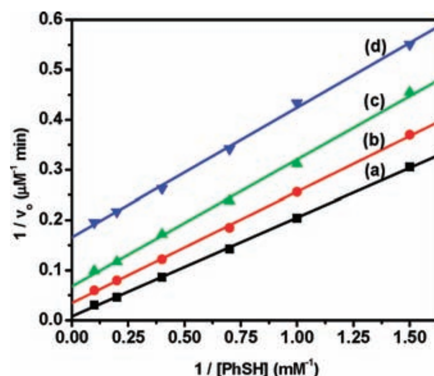

Figure 5. Lineweaver−Burk plots obtained for inhibition of the GPx activity catalyzed by compound **5** in the presence of PhSH at various concentrations of GTG. [GTG]: (a) 30 μM; (b) 50 μM; (c) 70 μM; (d) 90 μM. For each concentration of GTG, the cosubstrate (PhSH) concentration was varied, while keeping the concentration of H_2O_2 constant.

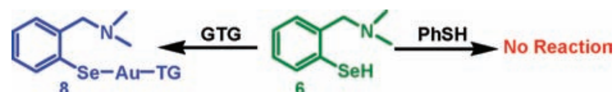

Figure 6. Reactivity of selenol **6** toward GTG and PhSH.

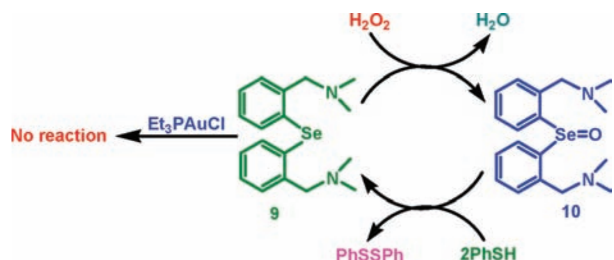

Figure 7. Mechanism for the catalytic reduction of H_2O_2 by the monoselenide **9** in the presence of PhSH. The formation of PhSSPh was followed by HPLC techniques, and oxidation−reduction reactions at the selenium center were confirmed by ^{77}Se NMR spectroscopy.

Effect of Gold(I) Compounds on the GPx-like Activity of Diaryl Monoselenide 9. The observations that inhibition of the GPx activity of **5** is due to the reaction of selenol **6** with gold(I) compounds are further supported by experiments with the monoselenide **9**, which does not produce any selenol species during the catalytic cycle. Although the GPx activity of compound **9** (18.4 μM·min^{-1}) at 250 μM concentration was found to be lower than that of the diselenide **5** (46.0 μM·min^{-1}) at 2.5 μM concentration, the activity of **9** was sufficient to follow the inhibition by Et_3PAuCl. It should be noted that compound **9** exhibits its GPx-like activity via oxidation of the selenium center, as shown in Figure 7. The oxidation of **9** by H_2O_2 produced the corresponding selenoxide **10**, which could be conveniently reduced back to the monoselenide **9** by thiols. The formation of selenoxide **10** was confirmed by isolation and spectral characterization of the product from the reaction of compound **9** with hydrogen peroxide. The ^{77}Se NMR experiments indicated that there was no selenol produced during the entire process.

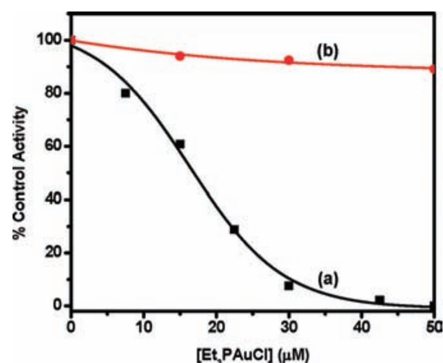

Figure 8. Effect of Et_3PAuCl on the GPx activity of compounds **5** and **9**. The amount of inhibition is expressed as a percent of the control activity for compounds **5** (a) and **9** (b) in the absence of Et_3PAuCl. The initial rates (v_0) for the reduction of H_2O_2 by **5** (2.5 μM) and **9** (250 μM) in the absence of added inhibitor were 46.0 and 18.4 $\mu M \cdot min^{-1}$, respectively.

Interestingly, while the GPx activity of compound **5** was completely abolished at 50−60 μM Et_3PAuCl, the activity of the monoselenide **9** was almost unaffected by treatment with Et_3PAuCl up to a concentration of 60 μM (Figure 8). The reactivity of the monoselenide **9** toward gold compounds was further confirmed by ^{77}Se and ^{31}P NMR experiments. For example, the ^{77}Se NMR signal observed at 342 ppm for compound **9** was unaffected upon the addition of chloro(trimethylphosphine)gold(I). Similarly, the ^{31}P NMR signal observed for chloro(trimethylphosphine)gold(I) at −10.10 ppm was unchanged in the presence of compound **9** (Figures S14 and S49 in the Supporting Information). These experiments suggest that the monoselenide **9** does not react with gold(I) chlorides during the inhibition assay. Therefore, inhibition of the GPx activity of selenium compounds by gold(I) drugs can be ascribed entirely to the reaction between selenol and gold compounds to afford the corresponding gold selenolate species. The complete inactivation of **5** by Et_3PAuCl indicates that the high GPx activity of this compound is clearly due to the conversion of diselenide **5** to the corresponding selenol (**6**) and the activation of the selenol moiety by the tertiary amino substituent. Therefore, the gold-based inhibition can also be used to understand the role of selenol in the GPx-like catalytic mechanism.

Ligand-Exchange Reactions in Gold Selenolate Complexes. Our further studies on the reactivity of selenol **6** toward GTG and R_3PAuCl (R = Me, Et, or Ph) reveal that the gold(I) chlorides are much more reactive than GTG toward the selenol moiety. The reaction of **6** with GTG did not give any isolable product. This reaction afforded a pale-yellow insoluble material, which could not be characterized. On the other hand, the reactions of **6** with R_3PAuCl afforded the corresponding gold(I) selenolates (Figure 9), which were identified by ^{31}P and ^{77}Se NMR spectroscopy and mass spectrometric techniques (these data are included in the Supporting Information). For example, free Ph_3PAuCl exhibited a ^{31}P NMR signal at 33.3 ppm, which was shifted slightly downfield (37.5 ppm, in $CDCl_3$) for the gold(I) selenolate (**13**).[12] Similarly, the ^{77}Se NMR signal observed at 37 ppm for the selenol **6** showed considerable downfield shift (74 ppm, in $CDCl_3$) upon coordination with a gold(I) compound. Mass spectrometry was also found to be useful in identifying the products formed in the reactions. The mass spectrum of the product obtained by treating **6** with Ph_3PAuCl showed a peak at 674.0 ppm with the expected isotopic pattern, which can be ascribed to the gold(I) selenolate complex (**13**). Similarly, the formation of compounds **11** and **12** was also confirmed by NMR (^{31}P and ^{77}Se) and mass spectrometric techniques. The reaction of *N*-Boc-L-selenocysteine methyl ester[13] with Me_3PAuCl produced the corresponding gold selenolate complex (Figures S50−S54 in the Supporting Information), indicating that the reactivity of **6** toward gold(I) complexes is similar to that of the selenocysteine moiety in GPx.

Coffer et al. have previously shown that the reaction of serum albumin (Alb) with AUR produces a gold thiolate complex. They have also shown that thiols can displace either the albumin or the triethylphosphine moiety from the protein−gold complex, $AlbSAuPEt_3$.[14] Shaw et al. have reported an interesting observation that the reaction of Alb with the triisopropylphosphine analogue of AUR eliminates triisopropylphosphine, which, in turn, reacts with the protein−disulfide bond to produce the corresponding phosphine oxide and sulfide.[14b] Although such displacement reactions have not been reported for gold selenolate complexes of selenoenzymes,[15] the presence of thiols may affect the stability of gold selenolates. Therefore, we have studied the interactions of complexes **11**−**13** with thiols such as PhSH and DTT. Although compounds **11**−**13** are reasonably stable in solution, the ^{31}P and ^{77}Se NMR spectroscopic experiments suggest that some ligand displacement reactions take place in the presence of thiols.[15] When the reaction of selenol **6** (produced in situ from the reaction of the diselenide **5** with DTT) with Ph_3PAuCl was followed by ^{31}P NMR spectroscopy, the signal for Ph_3PAuCl (33.3 ppm) disappeared completely upon addition of the selenol **6**. The

(12) The ^{31}P NMR chemical shifts observed for trialkyl/arylphosphinegold selenolate complexes **11**−**13** are comparable with that of the triphenylphosphinegold(I) complex (36.8 ppm) derived from *o*-carborane. Canales, S.; Crespo, O.; Gimeno, M. C.; Jones, P. G.; Laguna, A.; Romero, P. *Dalton Trans.* **2003**, 4525–4528.

(13) *N*-Boc-L-selenocysteine methyl ester was synthesized by the following literature method. (a) Bhat, R. G.; Porhiel, E.; Saravanan, V.; Chandrasekaran, S. *Tetrahedron Lett.* **2003**, *44*, 5251–5253. (b) Phadnis, P. P.; Mugesh, G. *Org. Biomol. Chem.* **2005**, *3*, 2476–2481.

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(15) In the presence of GSH, the ligand displacement reactions may take place to produce gold glutathione complex $Au(SG)_2^-$.[3c] It has been reported that thiourea can replace both the triethylphosphine (PEt_3) and thioglucose ($SATg^-$) moieties from AUR. The eliminated triethylphosphine may be converted to the corresponding triethylphosphine oxide.[14a] A similar ligand displacement reaction was observed when DTT was added to the gold selenolate complex. However, when PhSH was present, the phosphine displacement reaction was found to be extremely slow. For example, the ^{31}P NMR signal observed for Ph_3PAuCl at 33.3 ppm in $CDCl_3$ was unchanged upon the addition of even a large excess (10 equiv) of PhSH. Similar results were obtained when the experiments were carried out in MeOH. Furthermore, the phosphine ligand displacement by PhSH was not observed after the formation of gold selenolate complexes (**11**−**13**), suggesting that the gold selenolate complexes having a Se−Au−P moiety can be obtained in the presence of thiols. Ahhmad, S.; Isab, A. A. *J. Inorg. Biochem.* **2002**, *88*, 44–52.

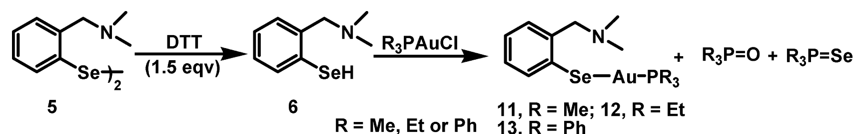

Figure 9. Reduction of diselenide **5** to produce selenol **6** and the reaction of **6** with R_3PAuCl (R = Me, Et, or Ph).

products obtained from this reaction exhibited a major peak at 37.5 ppm along with two minor peaks at 35.4 and 31.1 ppm. The major peak at 37.5 ppm can be assigned to the expected gold selenolate complex **13**, which is further confirmed by ^{77}Se NMR and mass spectral analysis (Supporting Information). The peak at 31.1 ppm indicates the formation of triphenylphosphine oxide ($Ph_3P{=}O$) during the reaction. This is due to the spontaneous oxidation of Ph_3P produced by a ligand displacement reaction. A control experiment involving the reaction of Ph_3PAuCl with DTT indicates that DTT does not replace the triphenylphosphine in Ph_3PAuCl, but the ligand displacement takes place only after formation of the gold selenolate complex (**13**). Interestingly, the third product obtained from this reaction was identified as triphenylphosphine selenide ($Ph_3P{=}Se$), which exhibited a peak at 35.4 ppm with the expected $^{31}P-^{77}Se$ coupling (J_{P-Se} = 727 Hz). The intensity of the signal at 35.4 ppm was increased upon the addition of an excess amount of DTT, indicating that the ligand-exchange reaction enhances formation of the $Ph_3P{=}Se$ species. A series of ^{31}P and ^{77}Se NMR experiments indicate that the attack of DTT at the gold center leads to the elimination of Ph_3P. The rapid reaction of Ph_3P with elemental selenium produces $Ph_3P{=}Se$. The elemental selenium present in the system is produced during the reductive cleavage of diselenide **5** by DTT to generate the active selenol **6**. Because the complexation reactions were performed with the in situ generated selenol **6** and gold chlorides, a minor amount of the eliminated trialkyl/arylphosphines by ligand displacement reactions reacts with the elemental selenium to produce trialkyl/arylphosphine selenide. The formation of triphenylphoshine selenide was confirmed independently by treating triphenylphosphine with elemental selenium under identical experimental conditions (Figures S59 and S60 in the Supporting Information). Similarly, compounds **11** and **12** undergo ligand displacement reactions to produce trimethylphosphine oxide and triethylphosphine oxide, respectively. Furthermore, the formation of trimethyl- and triethylphosphine selenide was also observed in these reactions.

Although DTT enhances ligand displacement reactions (Figure S26 in the Supporting Information), the stability of the gold selenolate complex is unaffected by PhSH. Interestingly, when complex **11** was treated with selenol **6**, the ^{77}Se NMR signal at 87 ppm for complex **11** disappeared completely to produce a new signal at 57 ppm, which can be ascribed to the formation of complex **14** (Figure 10).[16] The formation of compound **14** in this reaction was further confirmed by mass spectrometric techniques. The peak corresponding to the eliminated trimethylphosphine was not observed because the trimethylphosphine produced in this reaction was further converted to trimethylphosphine selenide and a trace amount of trimethylphosphine oxide (Figure 10). This indicates that inhibition of the GPx activity of compound **5** by R_3PAuCl is due to the formation of gold selenolate complexes **11**−**13** or the bis-selenolate complex **14**. However, the nature of the species formed in this reaction depends on the ratio of selenol to R_3PAuCl concentration. Therefore, during the inhibition experiments, at a lower concentration of R_3PAuCl, the bis-selenolate complex **14** is expected to be the predominant species because the reaction of 0.5 equiv of Me_3PAuCl with 1 equiv of selenol **6** produces **14** as the major product, whereas the formation of gold selenolate complexes **11**−**13** may dominate at higher concentrations of gold(I) chlorides. This is in agreement with the formation of a linear −S−Au−S− coordination during the inhibition of human glutathione reductase by compound **3**.[8b]

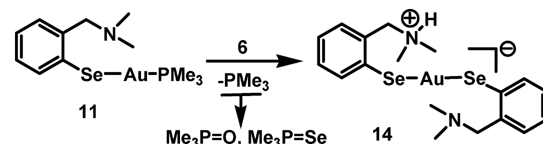

Figure 10. Reaction of gold selenolate **11** with selenol **6**. The trimethylphosphine eliminated from this reaction is converted to trimethylphosphine oxide and the corresponding selenide.

The reaction of trimethylphosphine complex **11** with Ph_3P produced the corresponding triphenylphosphine complex (**13**), indicating that the ligand displacement reactions can occur with different phosphines. In this phosphine-exchange reaction, two complexes **11** and **13** do not appear to be in equilibrium because the PMe_3 eliminated in this reaction is further oxidized to produce $Me_3P{=}O$ and $Me_3P{=}Se$ along with formation of the triphenylphosphine derivatives ($Ph_3P{=}O$ and $Ph_3P{=}Se$). The ^{31}P and ^{77}Se NMR spectroscopic experiments indicate that the phosphine-exchange reaction in complex **11** may take place via the formation of a trimethylphosphine−triphenylphosphine intermediate (**15**). This compound undergoes a further phosphine-exchange reaction in the presence of an excess amount of PPh_3 (5 equiv) to produce another intermediate (**16**) having $Au(PPh_3)_2^+$ as the counterion. The intermediate **16** is then dissociated to produce the gold(I) selenolate complex **13** (Figure 11).

When 0.5 equiv of triphenylphosphine was added to compound **11**, both the starting material (**11)** and the triphenylphosphine derivative (**13)** were detected by ^{31}P NMR and mass spectral studies (Figures S35−S37 in the Supporting Information). The addition of an excess amount of triphenylphosphine (5 equiv) to the reaction mixture led to the complete conversion of **11** to **13** with an increase in

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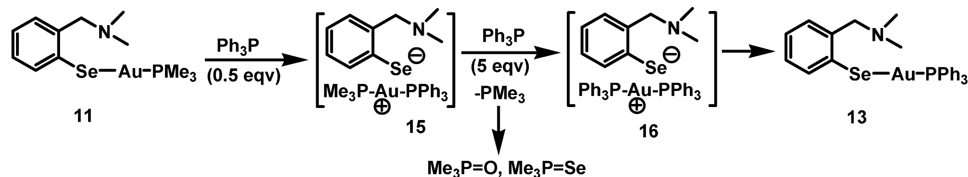

Figure 11. Phosphine-exchange reaction in complex **11** and the formation of complex **13**.

the intensity of the peak for $Me_3P{=}O$. The formation of **15** as an intermediate during the conversion of **11** to **13** can be rationalized from the line broadening in the ^{31}P NMR signals and the appearance of a new signal at 90 ppm in the ^{77}Se NMR spectrum. Although three-coordinate gold(I) phosphine complexes are known,[17] the mass spectral data indicate the formation of $[R_3PAuPR_3]^+$ during the ligand-exchange reaction (Figure 11). This is in agreement with previous reports that the formation of two-coordinate $[Ph_3PAuPPh_3]^+$ species can be isolated in the reaction between Ph_3PAuCl and Ph_3P in the presence of sterically bulky counterions (PF_6^-, BF_4^-, and NO_3^-).[18]

Conclusions

In summary, inhibition of the GPx activity by antiarthritic gold drugs has been studied by utilizing synthetic selenium compounds as enzyme mimics. The competitive nature of inhibition of the GPx activity of diaryl diselenide **5** by gold(I) drugs with respect to the peroxides and the absence of any such inhibition in the case of diaryl monoselenide **9** support the assumption that gold(I) compounds inhibit the GPx activity by reacting with the active selenol moiety to form a gold selenolate compound. Therefore, inhibition of the GPx activity by gold compounds can be used as a probe for identifying the involvement of a selenol in the catalytic mechanism of synthetic selenium compounds. The gold selenoate complexes having phosphine ligands undergo ligand displacement reactions to produce the bis-selenolate complex in the presence of selenols. In addition to the formation of trialkyl- and triphenylphosphine oxides, these ligand-exchange reactions lead to the formation of the corresponding phosphine selenides. Although gold(I) compounds inhibit the GPx activity in vivo, more research is warranted to determine whether gold(I) inhibition of GPx has any negative effect on the antioxidant defense mechanism.

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Supporting Information Available: Selected tables and all of the inhibition plots and ^{31}P and ^{77}Se NMR and mass spectra for the reactions of the thiols and selenols with gold(I) compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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