Novel gold(i) phosphine compounds inhibit HIV-1 enzymes

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The increasing incidence of human immunodeficiency virus (HIV) infection and the associated acquired immune deficiency syndrome (AIDS) mortality rates as well as the sometimes severe side effects of highly active anti retroviral therapy (HAART) warrants the continuous search for new, less toxic drug candidates. The anti-HIV activity (inhibition of reverse transcriptase-RT and protease-PR in direct enzyme assays) of eleven gold(i) phosphine compounds are reported here. Uptake of the compounds by peripheral blood mononuclear cells (PBMCs) was demonstrated by inductively coupled plasma atomic emission spectroscopy (ICP-AES) while the effect of the compounds on cell viability was assessed using flow cytometry with annexin V and propidium iodide (PI). Of the 11 gold compounds tested, 7 significantly ($p < 0.05$) inhibited RT activity at concentrations of 25 and 250 μM while 3 compounds significantly inhibited its activity at 6.25 μM. In the anti-protease assay, 4 of the compounds significantly inhibited the enzyme ($p < 0.05$) at 100 μM. All of the compounds were taken up by PBMCs (demonstrated by ICP-AES) and were non toxic to these cells at clinically tolerable concentrations. The potential of these novel gold(i) phosphine compounds as anti-HIV agents is therefore promising and worthy of further investigation.

Introduction

With more than 25 years elapsed since the discovery of HIV as the causative agent of AIDS, numerous investigators have dedicated enormous effort to finding promising drug leads (both synthetic and natural), to supplement existing treatment. A major success in the area of HIV/AIDS has been the use of HAART which has led to decreased morbidity and mortality. Drug resistance and the fact that treatment is not always well tolerated are some of the shortcomings of HAART e.g. the development of HIV strains resistant to AZT (3’-azido-3’-deoxylthymidine); one of the first potent anti-RT drugs. Resistance to other drugs in addition to AZT and the side effects associated with HAART necessitates the development of novel potential drug leads for use in new cocktail formulations or as stand-alone agents.

Gold-based drugs have a tremendous reputation in the treatment of many diseases e.g. rheumatoid arthritis with a resultant remission of the disease and have also been reported as having activity against microorganisms like HIV. In 1993, Okada and colleagues demonstrated the anti-HIV activity of aurothiogluco and aurothiomalate in cell-free and cell-based assays. These authors showed that inhibition of HIV by these compounds was through gold(i) ligand exchange of the reactive species bis(thiolato) gold(i) and acidic thiol groups exposed on viral surface proteins as well as by inhibition of reverse transcriptase in cell-free assays. Gold cyanide (Au(CN)$_2$), a common metabolite in patients treated with gold-based drugs was also reported by Tepperman et al. (1994) to have anti-HIV activity through inhibition of reverse transcriptase. Other anti-arthritic agents e.g. auranofin led to an increase in the CD4+ count of an HIV positive patient being treated for psoriatic arthritis while aurothiogluco was further reported to inhibit HIV-1 replication in latently infected OM10.1 and Ach2 cells. In 2001, Yamaguchi et al. used a mouse AIDS model to show the in vivo anti-HIV activity of gold sodium thiomalate. More recently, Bowman et al. (2008) showed that the addition of gold nanoparticles to a failed HIV drug resulted in inhibition of viral entry into TZM-bl cells as well as decreased secretion of viral p24 antigens. Recent findings by our group demonstrated inhibition of both RT and PR by a gold(iii) compound in direct enzyme inhibition assays. Gold-containing compounds therefore have potential as anti-HIV drugs and are worthy of further investigation.

Eleven novel gold(i) phosphine complexes and four of their ligands (starting material for producing gold-based drugs) were tested for potential inhibition of HIV enzymes. The use of phosphine gold compounds is supported by the fact that the presence of this group results in lipophilicity. Auranofin is an example of a phosphine-containing gold drug in clinical use which is taken orally notably because of its lipophilicity. Uptake of potential drugs into cells is very important for bioactivity; the injectable gold compound aurothiogluco (a non phosphine-containing complex) was shown to inhibit reverse transcriptase in cell free assays but together with its metabolites could not readily enter cells. Drug uptake is obviously important for effective inhibition of enzymes like RT in cell cultures and eventually in vivo. In general therefore, gold phosphine ligands are more lipophilic than...
non-phosphine analogues. Increases in lipophilicity enhance transport of the gold complex through cellular membranes and thus facilitate association of the gold compound with the intracellular active site. Earlier studies on structure activity relationships of 63 auranofin analogues demonstrated the importance of the phosphine ligand as derivatives lacking this moiety were significantly less active. The choice of gold(i) over gold(ii) compounds for example avoids the toxicity that is associated with the oxidizing power of gold(ii).

The 11 novel gold(i) phosphate complexes and 4 ligands were tested in cell free anti-RT and anti-PR assays to determine their HIV therapeutic value. The uptake of the compounds by PBMCs was confirmed by ICP-AES while toxicity to these same cells was assessed using flow cytometry.

Materials and methods

Compounds

The compounds tested were provided by chemists from the Project AuTEK consortium (Mintek and Harmony Gold, South Africa). Material utilized in this study will be referred to as either gold compounds/complexes (meaning those that have a gold atom) or ligands (referring to compounds with no gold atom). All complexes are gold(i) phosphate compounds and only differ because of the different ligands used in their preparation. The synthesis, characterization and purity of all compounds (Fig. 1) except compound 10 (auranofin) were reported on previously. Compound 10 was purchased from Biomol International L.P. (Pennsylvania, USA). The compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted in either assay buffer or culture media to a final DMSO concentration of ≤0.5% for experiments. The structures of the compounds and ligands as well as their molecular weights and designated numbers are shown in Fig. 1.

HIV-1 reverse transcriptase assay

The reverse transcriptase colorimetric assay kit from Roche Diagnostics (Mannheim, Germany) and recombinant HIV-1 RT (Merck Chemicals (Pty) Ltd Lab Supplies, South Africa) were used to test inhibition to RT. The enzyme (20 μl – 0.2U) was incubated with different concentrations of the gold compounds for 2 h and RT activity measured.

Quantification of anti-PR activity of compounds

This assay was performed according to procedures by Lam et al., using a fluorogenic substrate Arg-glu-(EDANS)-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Lys-(DABCYL)-Arg (Sigma, Missouri, USA) [where EDANS is 5-(2-aminoethylamino)-1-naphthalene sulfonate and DABCYL is 4'-dimethylaminobenzene-4-carboxylate]. This substrate is a synthetic peptide sequence that contains a cleavage site (Tyr-Pro) for HIV-1 protease as well as two covalently modified amino acids for the detection of cleavage. An aliquot of the substrate (16 μM, 49 μl) and 1 μl of HIV-1 PR solution (1 μg μl⁻¹; Bachem, Switzerland) were added to a reaction mixture in assay buffer in the presence or absence (untreated control) of compounds to a final reaction volume of 100 μl. Following a 1 h incubation at 37 °C in black 96 well assay plates (Corning Incorporated, Corning, New York, USA), the fluorescence intensity was measured at an excitation wavelength of 355 nm and an emission wavelength of 460 nm using a Synergy microplate spectrofluorometer [BioTek, Analytical and Diagnostic Products (ADP), South Africa]. Acetyl pepstatin was used as a positive control for inhibition of HIV-1 protease while the blank consisted of assay buffer only. Data was analysed using the Gen5™ software (ADP, South Africa) and the percentage inhibition calculated using the formula: 100 – [(Test reagent RFU/untreated control RFU) × 100)] where RFU = relative fluorescence units.

Compound uptake using inductively coupled plasma atomic emission spectrometry (ICP-AES)

Cell culture reagents were obtained from Highveld Biologicals (Sandringham, South Africa) unless stated otherwise. PBMCs were isolated by Ficoll gradient centrifugation and utilized in uptake and cytotoxicity assessment. Following isolation, PBMCs were resuspended in RPMI 1640 medium containing 2 mM l-glutamine and supplemented with 10% foetal bovine serum and antibiotics (10 mg ml⁻¹ penicillin G, 10 mg ml⁻¹ streptomycin sulfate, 25 μg ml⁻¹ fungizone and 1% gentamycin sulfate).

Soluble gold compounds were added to the experiment at a final concentration of 25 μM to 1 × 10⁶ cells ml⁻¹. The control wells contained cells and medium only. Following 7 days of incubation, the cells were washed twice with phosphate buffered saline (PBS) to remove non-absorbed material and treated with analytical grade nitric acid (10%) and heat (56 °C, 30 minutes). This process aids in disruption of proteins, lipids or any other macromolecules, which may interfere with the assay. Clarified cell lysates were analysed on a Liberty 110 ICP-AES spectrophotometer at 242.8 nm. Absorption at this wavelength indicates the presence of gold in the lyase and the emission intensity is directly proportional to how much of a particular gold compound was taken up as compared to the control sample. A gold standard (Alfa Aesar, Ward Hill, USA) was used for calibrating the instrument and as a reference for gold based on the emission wavelength of the standard in relation to experimental treatments.

Cell viability in the presence of gold compounds

Flow cytometry, using Annexin V and propidium iodide was performed according to the manufacturer’s instructions (Beckman Coulter, California, USA). Following 7 days of incubation (5% CO₂, 37 °C) of the gold compounds with PBMCs (1 × 10⁶ cells ml⁻¹), the cells were washed (500 × g, 5 minutes) with ice cold PBS and transferred to plastic flow
Fig. 1  Compounds assayed for anti-HIV-1 activity. \(1a\) = (2-diphenylphosphanyl-benzylidene)-phenethyl, \(1b\) = Benzyl-(2-diphenylphosphanyl-benzylidene)-phenethyl-amine gold(i) chloride, \(2a\) = \(N\)-\(N\),\(N\)-dimethyl-ethane-1,2-diamine, \(2b\) = \(N\)-\(N\),\(N\)-dimethyl-ethane-1,2-diamine gold(i) chloride, \(3a\) = 2-diphenylphosphanyl-benzyl)-phenethyl-amine, \(3b\) = 2-diphenylphosphanyl-benzyl)-phenethyl-amine gold(i) chloride, \(4a\) = \(N\)-\(N\),\(N\)-dimethyl-ethane-1,2-diamine, \(4b\) = \(N\)-\(N\),\(N\)-dimethyl-ethane-1,2-diamine gold chloride, \(5\) = Bis(diphenylphosphino)-1,2-diethylhydrazine di(gold chloride), \(6\) = Bis[bis(diphenylphosphino)-1,2-diethylhydrazine] gold chloride, \(7\) = Bis[chlorido(di-(4-methoxyphenyl)phosphine)-1,2-diethylhydrazine d(gold chloride)] (6), \(8\) = bis[chlorido(di-(4-methoxyphenyl)phosphine)-1,2-diethylhydrazine d(gold chloride)] (4), \(9\) = 2,3,4,6-tetra-O-acetyl-1-thio-\(B\)-\(O\)-glucopyranosato-S)-(1,3,5-triaza-7-phosphaadamantane) gold(i), \(10\) = (2,3,4,6-tetra-O-acetyl-1-thio-\(B\)-\(O\)-glucopyranosato-S)-(triethylphosphine) gold(i), \(11\) = (2,3,4,6-tetra-O-acetyl-1-thio-\(B\)-\(O\)-glucopyranosato-S)-(1,3,5-triaza-7-phosphaadamantane) gold(i) (+1) trifluoromethanesulfonate (-1). \(1a, 2a, 3a\) and \(4a\) are ligands for gold compounds \(1b, 2b, 3b\) and \(4b\) respectively. Mr = molecular weight.
than 50% and sometimes as high as 95.8%. The ligands significantly (p < 0.05) inhibited HIV-1 RT activity at concentrations of 25 and 250 μM. Three of the mentioned seven potential inhibitors (4b, 5 and 7) also exhibited significant (p < 0.05) inhibition at 6.25 μM (Fig. 2).

The inhibition of RT by all these compounds was greater than 50% and sometimes as high as 95.8%. The ligands (1a, 2a, 3a, and 4a) and other gold compounds; 6, 9, 10 and 11 had no effect on the activity of HIV-1 RT. Percentages were calculated relative to an untreated control sample of enzyme and reaction mixture only. A positive inhibitor for HIV-1 RT (2.4 units inhibited RT 97.3%) was used.

Inhibition of protease activity

Four of the complexes (4b, 6, 9 and 11) significantly (p < 0.05) inhibited HIV-1 PR with more than 50% inhibition at 100 μM (Fig. 3).

Uptake of gold compounds by PBMCs

All the gold compounds were taken up by PBMCs as shown in Table 1. Gold uptake by these cells was highest for compounds 4b and 8.

Results

The gold compounds inhibit reverse transcriptase activity

Of the 11 gold compounds tested, seven (1b, 2b, 3b, 4b, 5, 7 and 8) significantly (p < 0.05) inhibited HIV-1 RT activity at concentrations of 25 and 250 μM. Three of the mentioned seven potential inhibitors (4b, 5 and 7) also exhibited significant (p < 0.05) inhibition at 6.25 μM (Fig. 2).

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Discussion

The shortcomings of HAART have led to the continual search for potential drug candidates for use as anti-HIV agents. Eleven gold(I) phosphine compounds and four ligands were tested to determine their inhibitory effect on HIV-1 enzymes: RT and PR. Seven of the gold compounds inhibited HIV-1 RT while four of them inhibited HIV-1 PR. Cross-inhibition was observed for 4b, which inhibited both RT and PR. The compounds were taken up by PBMCs and were also non toxic to these cells.

Compound 10, which has been reported to have an inhibitory effect on HIV following an in vivo study where it was able to increase a patient’s CD4+ count,7 did not however inhibit RT nor PR in vitro in this study. This suggests that 10 probably acts by a mechanism other than inhibition of the enzymes tested here. In a different study, incubation of compound 10 with cells reportedly led to a dose-dependent suppression in tumour necrosis factor mRNA induction.23 Tumour necrosis factor is regulated by NF-κB and this transcription factor regulates HIV-1 gene expression in latently infected cells.8 It is therefore possible that compound 10 inhibits HIV by suppressing these transcription factors and would not have inhibited RT and PR in the cell-free assays performed here. Another possible mechanism could be that 10 causes modification of a surface component of the virus as has been reported for other gold compounds that were shown to inhibit HIV.5 The RT inhibitory gold compounds may be better inhibitors of the enzyme because chlorine (attached to the Au—see structures in Fig. 1) makes a better leaving ligand than 50% and sometimes as high as 95.8%. The ligands significantly (p < 0.05) inhibited HIV-1 RT activity at concentrations of 25 and 250 μM. Three of the mentioned seven potential inhibitors (4b, 5 and 7) also exhibited significant (p < 0.05) inhibition at 6.25 μM (Fig. 2).

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intercalate in the DNA molecule being formed in the reaction...

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reporting the inhibition of the RT enzyme by gold compounds...

structural role in many enzymes. Other authors have also...

effect observed in metal-based drugs since metals play a key...

showed to inhibit HIV-1 RT because of the dipyrazolium...

that is necessary for therapeutic activity.

ICP-AES was previously used in our laboratory for...

determined uptake of metals by mononuclear cells and has been reported by McKeage et al. (2000) for the uptake of drugs and gold phosphine complexes. As mentioned before, gold thioglucose has been shown to inhibit HIV-1 RT in...
more efficiently by PBMCs (Table 1) and significantly inhibits RT at all three concentrations tested and PR at 100 μM. However compound 8 was efficiently taken up but did not significantly inhibit both enzymes except for RT at 250 μM. It would be interesting to correlate uptake and inhibition of HIV in cell-based antiviral assays for 4b and all the other gold compounds that inhibited either of the enzymes. The presence of the phosphine ligands in these gold compounds and the associated lipophilicity probably enhanced the uptake of the compounds. Studies on the extent of lipophilicity of each compound compared to the specific uptake per compound would better correlate this. Concentrations at which compounds were able to inhibit HIV-1 enzymes were also non-toxic to PBMCs according to flow cytometry and annexin V (Fig. 4). The ligands caused the least toxic effect on the cells compared to the gold compounds. All the gold compounds were tested 6 months after synthesis and were freshly prepared prior to activity screening. The possibility of the compounds losing biological activity over time and under different storage conditions form part of ongoing investigations.

Conclusion

All 11 novel gold(I) phosphine compounds reported here could be taken up by peripheral blood mononuclear cells and were non toxic to these cells. Seven of the gold compounds inhibited reverse transcriptase, while four displayed anti-protease, activity. The phosphine ligands assayed did not exhibit appreciable inhibition of either enzyme confirming the fact that the presence of the gold metal enhanced antiviral activity. Since uptake generally correlates to biological activity, these compounds show promise as potential inhibitors of these HIV-1 enzymes in cell culture and potentially in vivo and thus warrant further analysis.

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