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HIV therapeutic possibilities of gold compounds

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Abstract Highly active antiretroviral therapy (HAART) has resulted in decreased mortality and morbidity from the acquired immune deficiency syndrome caused by the human immunodeficiency virus (HIV). Drug resistance and toxicity of HAART has led to the search for novel inhibitors of HIV infection. Gold-based compounds have shown promising activity against a wide range of clinical conditions and microorganism infections including HIV-1. A typical example is auranofin which resulted in an elevated CD4+ T-cell count in an HIV patient being treated for psoriatic arthritis. In addition, reports exist on gold-based inhibitors of reverse transcriptase (RT), protease (PR) and viral entry of host cells. These and other characteristics of gold-based HIV drugs are reviewed here.

Keywords Gold · Inhibitors · HIV · AIDS

Abbreviations

$(H_2TMPyP)^{4+}$	Meso-tetrakis(*N*-methlypyridinium-4-yl)porphyrin
AIDS	Acquired immune deficiency syndrome
$Au(CN)_2$	Dicyanogold
AuTG	Aurothioglucose
AuTM	Aurothiomalate
$[bpzmH_2]$ $[AuCl_4][Cl]$	Tetra-chloro-(bis-(3,5-dimethlypyrazolyl) methane)gold(III) chloride
CCR5	Chemokine receptor 5
CD25	Cluster of differentiation 25
CD4	Cluster of differentiation 4
CD45	Cluster of differentiation 45
CD8	Cluster of differentiation 8
CEM, MT-4, OM10.1, Ach2	Cell line abbreviations
Cys	Cyteine
DNA	Deoxyribonucleic acid
GST	Gold sodium thiomalate
H_2dcbpb	4,5-Dichloro-1,2-bis(2-(4-tert-butylpyridine) carboxamido)benzene
H_2salen	*N*,*N*′-ethylenebis(salicylideneimine
HAART	Highly active antiretroviral therapy
$HAuCl_3$	Hydrogen gold chloride
HIV	Human immunodeficiency virus
IL	Interleukin
IL-2R	Interleukin-2 receptor
NF-κB	Nuclear factor kappa B
NP	Nanoparticle
PBMCs	Peripheral blood mononuclear cells
PR	Protease
RNA	Ribonucleic acid

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RT	Reverse transcriptase
TG	Thioglucose
TNF-α	Tumor necrosis factor-alpha
TPP	*meso*-Tetraarylporphyrin

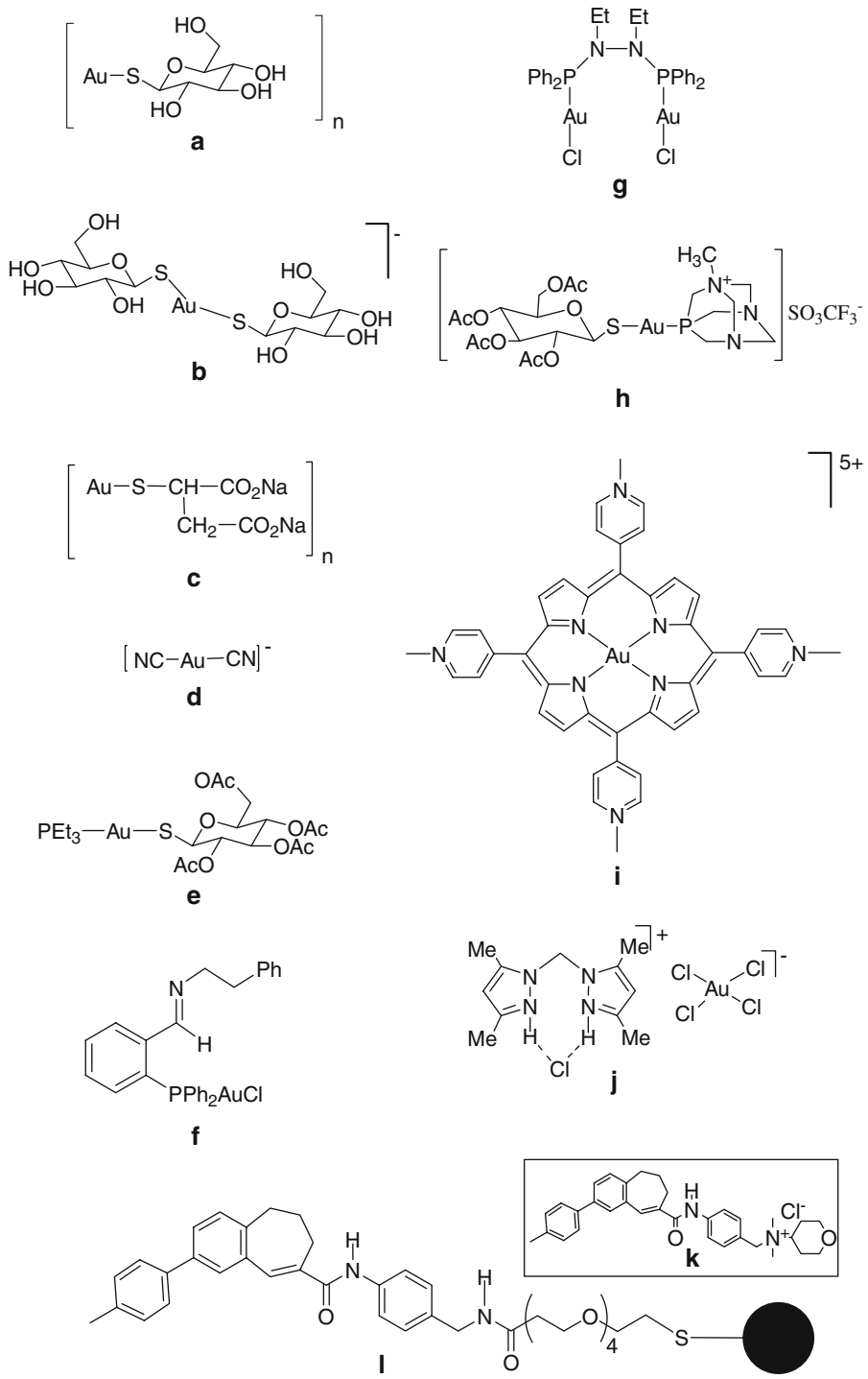
Introduction

A major success story of HIV/AIDS is the effect of HAART on morbidity and mortality rates (Pallela et al. 1998). Side effects due to anti-retroviral drugs and the occurrence of drug resistant mutants are some of the reasons for the continuous search for novel treatments. Most drugs against HIV are organic compounds e.g. 3′-azidothymidine, but there is no doubt that metal-based compounds could offer alternatives. Metal-based drugs are synthetic compounds containing a metal and a suitable ligand, which is usually an organic molecule. The presence of the metal ion enhances the activity of the organic molecule. This is possibly due to the stabilization of the drug by coordination to the metal ion; a concept known as metal-drug synergism (Navarro 2009). Different metals such as platinum, vanadium (Sadler and Guo 1998; Sadler et al. 1999; Suerbaum and Michetti 2002; Sun et al. 2007), ruthenium, gold and rodium amongst many others have been shown to have medicinal properties when complexed with organic ligands. The ligand is believed to serve as a vehicle for transporting the metal to its active site (Shaw 1979, 1999).

Gold based preparations have been used in medicine since 2500 BC in China (Sutton 1986; Fricker 1996a, Huaizhi and Yuantao 2001). The history of gold compounds as drugs is prominently depicted in their successful use as anti-rheumatoid arthritic agents e.g. solganol (aurothioglucose), myocrisin (sodium aurothiomalate) and auranofin (Fig. 1a, c and e, respectively). These compounds have been reported to not only slow the evolution of rheumatoid arthritis (Sutton 1986; Best and Sadler 1996; Ahmad 2004) but also to have promising anti-cancer activity (Fricker 1996b) and activity against a broad spectrum of microorganisms (Leibfarth and Persellin 1981). Gold-based compounds, reportedly, also have anti-malaria qualities (Navarro et al. 1997) but more central to this review is the fact that gold compounds have been implicated as anti-HIV agents (Blough et al. 1989; Okada et al. 1993; Tepperman et al. 1994; Shapiro and Masci 1996; Traber et al. 1999; Yamaguchi et al. 2001; Sun et al. 2004; Fonteh et al. 2009; Fonteh and Meyer 2009).

Fig. 1 Structures of gold compounds with HIV-1 inhibitory activity: **a** Solganol = aurothioglucose, **b** bis(thioglucose) gold(I), **c** Myocrisin = sodium aurothiomalate, **d** dicyanogold(I), **e** Auranofin (Radiura), **f** benzyl-(2-diphenylphosphanyl-benzylidene)-phenetyl-amine gold(I)chloride, **g** bis(diphenylphosphino)-1,2-diethylhydrazine di(gold chloride), **h** (2,3,4,6-tetra-*O*-acetyl-1-thio-*B*-D-glucopyranosato-S-)(1, 3, 5-triaza-7 phosphaadamantane)gold(I) (+1) trifluoromethanesulphonate(−1), **i** porphyrin complex Au(TMPyP)Cl_5, **j** Tetra-chloro(bis(3,5-dimethylpyrazolyl)methane)gold(III) chloride, **k** TAK-779, derivative of nanoparticle-based compound: (SDC-1721)-NP), **l** (SDC-1721)-NP): nanoparticle-based compound. Compounds **a**–**h** gold(I) compounds, **i** and **j** gold(III) compounds while **k** nanoparticle based gold compound while l is a derivative of the gold nanoparticle-based compound (cf. Bowman et al. 2008; Fonteh et al. 2009; Fonteh and Meyer 2009; Fricker 1996a, b; Sun et al. 2004)

Though the anti-HIV activity of gold compounds is not well documented as the anti-arthritic and anti-tumour activity, promising results have been obtained from both clinical and laboratory studies suggesting that these compounds may play a role in HIV/AIDS therapy. The earliest reports of the effect of gold on HIV were in the late 80s by Blough et al. (1989) who demonstrated the ability of gold containing aliphatics to inhibit RT. Most of the gold-based compounds that have been reported to have anti-HIV activity have gold in its +1 oxidation state (gold(I)). Gold(I) compounds have minimal toxic effects as compared to gold(III) compounds. This is due to the fact that gold(I) is thermodynamically more stable than gold (III) with most gold(III) complexes being strong oxidizing agents which are easily reduced to gold(I). Biologically occurring reductants such as thiols reduce gold(III) to gold(I), making gold(III) compounds generally very toxic (Fricker 1996a). That notwithstanding, there is evidence of gold(III) compounds with anti-HIV activity and limited toxicity (Sun et al. 2004; Fonteh et al. 2009). A judicious choice of hard donor (e.g. N or O) containing ligands normally produce relatively stable gold compounds in their +3 oxidation state, which in turn could lead to minimal or no toxicity to normal cells. For instance, an anti-HIV gold(III) compound (Au(TPP))Cl (TPP = *meso*-tetraarylporphyrin), prepared by using a porphyrin-based ligand was reported to be stable in physiological buffer with no significant decomposition in the presence of excess glutathione (Sun et al. 2004). In our recent findings (Fonteh et al. 2009), a



gold(III) compound stabilized by a pyrazolyl ligand showed dual activity against two HIV enzymes with insignificant toxicity to peripheral blood mononuclear cells (PBMCs).

Another recent report comments on a nanoparticle-based gold compound that transformed a weakly binding and biologically inactive small molecule into a multivalent conjugate that effectively inhibited

HIV-1 fusion to human T cells (Bowman et al. 2008). This report seeks to highlight the importance that gold compounds may have in combating HIV and AIDs. Gold-based compounds that have shown activity against HIV, together with their postulated mechanisms of action, are discussed below. The structures of the gold compounds discussed herein are presented in Fig. 1 and their mechanistic and inhibition information summarized in Table 1.

Gold(I) compounds

Aurothioglucose

Aurothioglucose (AuTG; Fig. 1a), known for its clinical use in the treatment of rheumatoid arthritis, was evaluated for anti-HIV activity by Okada et al.(1993). This compound and other gold containing aliphatics had earlier been reported by Blough et al. (1989) at an international conference on AIDS to be effective against HIV-1 RT in cell lysates. Similarly, Okada et al. (1993) demonstrated that AuTG was not only capable of inhibiting RT in a cell free assay but that it was an effective inhibitor of HIV-1_{NL4-3} infectivity. AuTG is known to form reactive gold-thiol intermediates upon addition of thiol ligands that are capable of interacting with thiol groups of cysteinyl residues on the surface of proteins (Shaw 1979, 1999). In their work, Okada et al. (1993) demonstrated that the reactive intermediate which is bis(thiogluocse)gold(I)-bisAuTG (Fig. 1b) can undergo ligand exchange with thiol groups exposed on the surface of proteins. BisAuTG, which is formed by reacting a molar equivalent amount of AuTG and 1-thio-β-D-glucose, was able to protect MT-4 cells from infection and lysis by HIV-1_{NL4-3} (Okada et al. 1993). Inhibition of viral entry or infectivity was reportedly through its reaction with Cys^{532} on gp160, a viral coat protein. BisAuTG was much more active than AuTG but unfortunately, lacked activity against more virulent strains of HIV. This lack of activity was presumably because of the differences in the envelope glycoproteins of the viruses (Okada et al. 1993). Given that HIV-1_{NL4-3} has a unique cysteine residue close to the amino terminus of its gp41 envelope glycoprotein, the reported inhibition was therefore as a result of the association of gold(I) with thiol groups on the surface proteins of the viral envelope via the ligand exchange process mentioned earlier. Therefore not only was bisAuTGs' inhibition of cell free RT confirmed, it was concluded that its antiviral activity was due to inhibition of viral infectivity.

In 1999 Traber et al. also demonstrated that AuTG significantly decreased the secretion of p24 antigen from latently infected OM10.1 and Ach2 cells (latency was maintained by adding 20 μM of AZT to infected cells and excluding it 2 weeks prior to experimentation). It is interesting that thioglucose (TG) and hydrogen gold chloride ($HAuCl_3$), the reactants from which AuTG is prepared, were inactive. AuTG significantly decreased p24 secretion from OM10.1 and Ach2 cells at 10 μM ($P < 0.01$) and 25 μM ($P < 0.02$), respectively. In addition, the compound inhibited tumor necrosis factor-alpha (TNF-α) induced HIV-1 replication in the latently infected OM10.1 and Ach2 cells.

Prevention of NF-κB binding to DNA in vitro was evidently responsible for the inhibition of HIV-1 replication by AuTG (Traber et al. 1999). Traber et al. suggested that the gold ions in AuTG either blocks the DNA binding activity of NF-κB or that the gold compounds were able to change the redox status of the cell and therefore prevent activation of NFκB. Alternatively Au(I) could also inhibit NF-κB-DNA binding by oxidizing the protein thiols associated with Zn instead of replacing them in vitro (Yang et al. 1995).

The concentrations that were used (Traber et al. 1999) were clinically relevant as they were similar to the concentration of gold in the synovium and blood of patients on chrysotherapy (Mascarenhas et al. 1972; Yoshida et al. 1998; Stern et al. 2005).

From the studies discussed above, AuTG was shown to inhibit HIV by prevention of viral entry through its reactive intermediate AuTG (Okada et al. 1993) and also by decreasing the secretion of p24 through prevention of NF-κB binding to DNA (Traber et al. 1999).

Traber et al. (1999) demonstrated the accumulation of metal gold (using electron microscopic examinations) within OM10.1 cells as a result of incubating them with AuTG. The decrease in p24 secretion from these cells may have resulted from the activity of a metabolite of AuTG and not AuTG itself, especially because gold-based drugs have been reported to be prodrugs (Shaw 1999). This is worthy of consideration because as reported by Tepperman et al. (1994) and

Table 1 Summary of the compounds that have inhibitory activity against HIV-1, mechanism of inhibition and some general comments

Gold compound	Inhibition/prevention of	Assay type	Mechanism of inhibition	References
Aurothioglucose and other gold containing aliphatics	HIV reverse transcriptase inhibition	Inhibition of RT in cell lysates (CEM cells) i.e. direct enzyme inhibition	Inhibits initiation and elongation of DNA chains by HIV reverse transcriptase	Blough et al. (1989)
Aurothioglucose and Bis(thioglucose) gold(I)[a] complex	Reverse transcriptase	Cell free culture supernatant	Association of gold(I) with thiol groups on the surface of viral envelope proteins via ligand exchange reactions	Okada et al. (1993)
	Viral entry and cytopathic effect of HIV-1_{NL4-3}	Cell culture: CEM and MT-4 cells	Modification of surface component of the virus by causing the release of gp120 preventing infection	
Aurothioglucose	p24 secretion from latently infected cells	Cell-based (OM10.1 and Ach2 cells)	Inhibits DNA binding of NF-$\kappa\beta$ (a transcription factor required by HIV-1)	Traber et al. (1999)
Aurothiomalate and bis(thiomalate)gold(I)[a]	Cytopathic effect of an HIV-1_{NL4-3}	Cell culture: CEM and MT-4 cells	Bis(thiomalate) was less active than bis(thioglucose) gold(I)	Okada et al. (1993)
Aurothiomalate	Gag expression, elevation of IL-2 levels, and increase of cell surface markers like CD4	Cell-based (mouse spleen cells)	Restored cytokine expression and CD4+ cell production in a mouse AIDS (MAIDS) model	Yamaguchi et al. (2001)
Dicyanogold	HIV-1 RT	Cell-based on H9 cell culture supernatant and lysates	Easily taken up by cells using the "sulfhydryl shuttle" and possibly interferes with the DNA binding activity of reverse transcriptase	Tepperman et al. (1994)
Auranofin	Possible immunomodulation, RT inhibition	Cell based	Increased the CD4+ count of an HIV patient being treated for psoriatic arthritis	Shapiro and Masci (1996).
Eleven gold phosphine compounds	RT and/or PR	Cell free/direct enzyme	Possible inhibition of DNA binding ability of reverse transcriptase and ligand exchange reactions in the case of PR inhibition	Fonteh and Meyer (2009)
$Au^{III}(TMPyP)Cl_5$[b] {$(H_2TMPyP)^{4+}$ = meso-tetrakis(N methlypyridinium-4-yl)porphyrin}	RT	Direct enzyme assay	HIV-1 RT inhibition by $(Au^{III}(TMPyP))Cl_5$ is related to its being bound to connection domain sequence 398-407 (WETWWTWYWQ). Also possibly due to binding to certain sites near the active site and linked to possible DNA intercalation	Sun et al. (2004)

Table 1 continued

Gold compound	Inhibition/prevention of	Assay type	Mechanism of inhibition	References
Tetra-chloro-(bis-(3,5-dimethlypyrazolyl) methane)gold (III) chloride[b]	RT and PR	Direct enzyme assay	RT inhibition involved only tetra-chloro-(bis-(3,5-dimethlypyrazolyl)methane)gold (III) chloride. The auric acid from which it was synthesized showed no inhibition. Inhibition of PR, however, seemed to involve both auric acid and tetra-chloro-(bis-(3,5-dimethlypyrazolyl)methane)gold (III) chloride	Fonteh et al. (2009)
(SDC-1721)-NP	Prevents viral entry and decrease p24 secretion	Cell based: TZM-bl cells and PBMCs	Entry inhibitor, possibly functions as a CCR5 antagonist	Bowman et al. (2008)

[a] Active component of aurothioglucose

[b] Gold(III) compounds

Zhang et al. (1995), AuTG as a gold compound does not readily enter cells and therefore is not able to reach the intracellular concentrations necessary to inhibit intracellular RT and even RT within virions (Okada et al. 1993). Inhibition of RT using AuTG has previously been reported in cell free assays (Blough et al. 1989). AuTG (in the form of bisAuTG) may still be effective as an inhibitor in early infection as it may prevent the virus from invading cells through the association of gold(I) with the thiol groups on the surface proteins of the viral envelope.

Aurothiomalate

Another important compound is aurothiomalate (AuTM) also known as gold sodium thiomalate (GST; Fig. 1c). This is also an anti-arthritic agent that has been shown to have anti-HIV effects (Okada et al. 1993; Yamaguchi et al. 2001). The reactive intermediate according to Okada et al. was bis(thiomalate)gold(I) which protected MT-4 and CEM cells against viral cytopathic effect but inhibition was to a lesser extent than that of bisAuTG (the reactive intermediate of AuTG).

Weekly treatment of LP-BM5 murine leukemia virus-infected mice with AuTM demonstrated its immunomodulatory effect (Yamaguchi et al. 2001). LP-BM5 murine leukemia virus causes a disease in mice that presents as immunosuppression and lymphoproliferation with features similar to AIDS. There was an induction of interleukin (IL)—2 production on the third and fifth day but less on the seventh day (18.1 ± 1.6, 34.5 ± 2.6, and <0.2 U/ml, respectively). The expression of the CD25 marker for IL-2R increased in the AuTM treated mice to 33% compared to 13% in the untreated mice. The mice survived longer, had less cervical lymph node swelling and generally had fewer abnormalities in the expression of cell surface markers such as CD4, CD8a and CD45R/B220 on their spleen cells.

Clinical trial reports of IL-2 treatment of HIV infected patients have shown that intermittent infusion led to substantial and sustained increases in CD4 counts without any increases in the plasma viral load in the long term (Kovacs et al. 1995). Cells that participate in eliciting immune responses, communicate via interleukins and cytokines (Miller 2002). These soluble proteins do not only serve as messengers but can also influence disease progression.

Chrysotherapy has been shown to reduce or regulate the production of other cytokines such as IL-6 and IL-8 in serum (Madhok et al. 1993); monocytes (Crilly et al. 1994), macrophages (Yanni et al. 1994), and synovial cells (Seitz et al. 1992; Loetscher et al. 1994; Yoshida et al. 1998). Yamaguchi et al. also observed that spleen cells from five HIV infected mice (not treated with AuTM) had gag specific bands, indicating the presence of viral DNA (i.e. 100% positivity). However, gag specific bands were detected in only 3 of 5 mice (60%) that had been treated with AuTM indicating a decrease in HIV-1 gene expression on treatment.

AuTMs' modulation of the immune system, with a resultant decrease in gag expression, increase in IL-2 secretion and expression of surface markers such as CD4+ in LP-BM5 murine leukemia virus-infected mice (Yamaguchi et al. 2001) points to a role for gold-based compounds in the stimulation of an immune response against HIV-like viruses and thus HIV.

Dicyanogold(I)

Spurred by findings that chrysotherapeutic drugs do not stay in circulation in patients to any significant extent and that the mechanism of action of gold compounds in these patients was not known, Tepperman et al. (1994) investigated the presence of gold containing metabolites in patients' biological fluids. They identified dicyanogold ($Au(CN)_2$)—(Fig. 1d) to be a common metabolite in patients on three different gold drugs (auranofin, AuTG, and AuTM). It is, however, important to note that the occurrence of dicyanogold was reported for these three gold(I) compounds and may not necessarily be a generality for all gold compounds.

These authors (Tepperman et al. 1994) went onto investigate the effect of dicyanogold on HIV using an H9 cell line, which supports the replication of the virus. Using this cell line, the authors were able to prove the uptake of gold ions from dicyanogold as well as monitor the intracellular activity of RT after infection and subsequent treatment with the compound. Non cytotoxic concentrations of dicyanogold(I) showed a 50% decrease in RT activity (Tepperman et al. 1994). The uptake studies elucidated here are crucial for cell based in vitro activity and eventually in vivo activity. As mentioned earlier, some gold compounds that inhibited HIV-1 RT in direct enzyme assays in vitro could not be taken up by cells such that bioactivity assessment was impossible (Tepperman et al. 1994, Zhang et al. 1995).

Ligand exchange reactions with sulfhydryl groups on enzymes (Allaudeen et al. 1985), has been reported to be the mechanism by which some gold compounds inhibit these enzymes. This could be the mechanism by which dicyanogold(I) inhibited RT in the H9 cells mentioned above.

Auranofin

Musculoskeletal disorders occur commonly in HIV patients and manifests due to host responses to infection, direct T cell dysfunction and immune mediated arthritis e.g. psoriatic arthritis (Wanchu 2003). Gold compounds such as auranofin (Fig. 1e) have been shown to result in the remission of rheumatoid arthritis and psoriatic arthritis. In 1996, observations made from clinical data of an HIV+ patient treated for psoriatic arthritis with auranofin showed a significant increase in CD4+ lymphocyte count (Shapiro and Masci 1996). Because natural progression of HIV infection is marked by a falling CD4+ count and considering that the patient was not on HIV medication; the assumption was that auranofin must have caused the improvement in the patients' condition. Shapiro and Masci (1996) showed that there was an increase in the patients CD4+ count from 160/mm^3 to 390–400/mm^3 following 5 months of auranofin therapy while the HIV-RNA copies dropped to almost 1,000 copies (lower limit of detection of the assay used). The authors suggested the possibility of auranofin inhibiting viral replication in such a way that the proliferating cells escaped the cytopathic effect and other negative responses caused to the immune system by the virus. Immune modulation of cytokines such as IL-2, TNF-α and other cytokines which are usually altered in AIDS, may have been restored. In addition, considering that patients on chrysotherapy (auranofin) tend to produce $(Au(CN)_2)^-$ (Fig. 1d) as a metabolite, we suggest that the inhibitory effect of this gold metabolite on RT through ligand exchange reactions could also have led to remission of the AIDS status of the patient. This report clearly demonstrates the clinical effect that auranofin had on the HIV/AIDS status of the patient in question. Notably, overall wellness of

the patient was restored upon treatment with auranofin.

Inhibition of HIV RT and PR by novel gold(I) phosphine complexes

Fonteh and Meyer (2009), demonstrated the inhibitory activity of eleven gold(I) phosphine compounds (Fig. 1f–h are representative compounds) against HIV-1 RT and HIV-1 PR. Seven of the tested compounds significantly inhibited HIV-1 RT activity with inhibitions ranging from 51.5 to as high as 95.8%. Four of the mentioned compounds inhibited HIV-PR with the highest inhibition being 72.6% while four ligands from which some of the complexes were synthesized had no significant inhibitory effect, especially, in the case of RT inhibition. The presence of gold metal in the complex must have resulted in the enhanced activity observed for these compounds. In addition to their inhibitory effects, the compounds were non toxic to immune system cells. Equally important is the fact that they could be taken up by cells probably because of the phosphine groups which are lipophilic, a characteristic that enhances compound uptake across cell membranes.

Gold(III) compounds

The literature recounted so far shows inhibition of HIV-1 by gold(I) compounds. The limitation of gold(III) compounds for use in drug development stems from the fact that gold(III) compounds tend to be thermally unstable and are reduced in physiological conditions. Gold(III) also tends to be toxic (Parish and Cottrill 1987). Nevertheless, a few gold(III) compounds have been reported to inhibit HIV. Gold(III) is isoelectric with platinum(II) and forms square planar compounds similar to cisplatin, a successful anticancer drug. As a result gold(III) compounds have been thought to have therapeutic effects similar to cisplatin. Research on gold(III) compounds as potential anticancer agents has been reported (Saqqioro et al. 2007; Keter et al. 2008; Angela et al. 2008). There are a few examples of gold(III) compounds that have been reported to show activity against HIV. These include [Au(salen)]Cl (H_2salen = *N*,*N*′-ethylene bis(salicylideneimine), [Au(dcbpb)]Cl (H_2dcbpb = 4,5-dichloro-1,2-bis(2-(4-tert-butylpyridine)carboxamido) benzene), [Au(TMPyP)]Cl_5 {$(H_2TMPyP)^{4+}$ = *meso*-tetrakis(*N*-methlypyridinium-4-yl)porphyrin} (Sun et al. 2004) and tetra-chloro-(bis-(3,5-dimethlypyrazolyl)methane)gold(III) chloride (Fonteh et al. 2009).

Au^{III}(TMPyP)Cl_5 {$(H_2TMPyP)^{4+}$ = meso-tetrakis(*N*-methlypyridinium-4-yl)porphyrin}

A porphyrin complex, [Au^{III}(TMPyP)]Cl_5 (Fig. 1i) is prepared from reacting *meso*-tetrakis(*N*-methlypyridinium-4-yl)porphyrin with $HAuCl_4 \cdot 4H_2O$ (Gibbs et al. 1988). The choice of porphyrin is informed by the fact that these compounds have been shown to stabilize gold(III) hence giving them stability in the biological *milieu*. [Au^{III}(TMPyP)]Cl_5 was found to be a successful inhibitor of HIV-1 RT in vitro with an effective inhibitory concentration at micromolar level (IC_{50} = 0.31 μM). At 6 μM, inhibition of RT of up to 70% was recorded (Sun et al. 2004). The presence of the gold atom was shown to be critical for the observed RT inhibition since all the ligands were largely inactive. The authors also used $KAuCl_4$ for comparison and only 48% inhibition was noted compared to 70% inhibition for [Au^{III}(TMPyP)]Cl_5. This further exemplifies the requirement for an organic molecule (ligand) in metallodrugs in enhancing activity. No significant cytotoxicity was observed when healthy human T-cells and fibroblasts were used as models for toxicity determination using [Au^{III}(TMPyP)]Cl_5. Further UV/Vis absorption titration analysis revealed that the HIV-1 RT inhibition by [Au^{III}(TMPyP)]Cl_5 is related to it being bound to connection domain sequence 398-407 (WETWWTWYWQ; Argyris et al. 1999). This is in agreement with the fact that gold(III) porphyrin binds to certain sites near the active site of the enzyme. The type of inhibition exhibited by these gold(III) porphyrin compounds is also linked to its possible intercalation between nucleotide base pairs of DNA (Gibbs et al. 1988).

Similar HIV inhibitory results were reported by Che (2004) in their work involving gold(III) complexes of porphyrins, schiff-bases, bis(pyridyl)carboxamide and bis(pyridyl)sulfonamides-based ligands. In effect therefore, there is an increased chance of gold(III) compounds with a porphyrin ligand to inhibit HIV-1 RT presumably through DNA intercalation, thus preventing the enzyme from catalyzing the conversion of viral RNA to cDNA and thus interrupting its life cycle.

Tetra-chloro-(bis-(3,5-dimethlypyrazolyl)methane) gold (III) chloride

Our recent findings showed that tetra-chloro-(bis-(3,5-dimethlypyrazolyl)methane)gold(III) chloride, [bpzmH_2][$AuCl_4$]Cl (Fig. 1j) inhibits both RT and PR and could therefore serve a dual role as part of possible HAART regiments (Fonteh et al. 2009). Inhibition of RT of up to 86.5% was observed for this compound at 5 μM and 100% at 250 μM. Since [bpzmH_2][$AuCl_4$][Cl] is a salt having $AuCl_4^-$ as the counterion, $HAuCl_4 \cdot 4H_2O$ (auric acid) was tested to investigate its effect on RT. Whereas auric acid alone did not show any significant effect, [bpzmH_2][Cl][$AuCl_4$] showed significant RT inhibition. On the other hand, the inhibition of PR seemed to involve both the auric acid and the compound. Inhibition of PR by [bpzmH_2][Cl][$AuCl_4$] was, however, significantly higher than that of the auric acid. Ligand exchange reactions with sulfhydryl groups on the surface of the enzymes could be responsible for this inhibition. Mechanistic studies are currently underway to further probe the compounds' exact mode of action.

Nanoparticle-based gold compounds

SDC-1721 nanoparticle (SDC-1721)-NP)

Nanotechnology is at present experiencing a rapid growth period with potential advances in the biomedical field under investigation (Caruthers et al. 2007). Nanoparticles are comparable in size to proteins and present multiple protein binding ligands that may be effective at disrupting the protein:protein interactions that drive disease pathogenesis (Bowman et al. 2008). Gold nanoparticles offer a multivalent binding strategy which confers many advantages. This has been demonstrated with gold nanoparticles which were shown to transform a weakly binding and biologically inactive small molecule (SDC-1721) into a multivalent gold nanopaticle therapeutic agent that effectively inhibited HIV-1 fusion to human T cells (Bowman et al. 2008). This was achieved by employing mercaptobenzoic acid modified gold particles (with proposed empirical formular being [$Au_{144}(SC_6H_4COOH)_{52}$]) resulting in the gold nanoparticle therapeutic agent. The particles have been likened to proteins and dendrimers in that they are atomically precise and monodispersed nanoscale molecules (Bowman et al. 2008). SDC-1721 is a derivative of TAK-779 (Fig. 1k) which is a CCR5 antagonist with a quaternary ammonium salt essential for high affinity binding and effective inhibition of HIV. TAK-779 has been reported to inhibit HIV-1 replication in viral infected PBMCs with an IC_{50} of 10 nM. In addition, IC_{50} values recorded for TAK-779 against four different CCR5-tropic viral isolates were between 1.6 and 3.7 nM (Baba et al. 1999) and 20 nM in a CCR5-tropic isolates similar virus, JR-FL (Safarian et al. 2006). The quaternary ammonium salt of TAK-779, however, resulted in poor pharmacological action and thus the need to search for alternative CCR5 antagonists.

The inactive derivative (SDC-1721) was synthesized, without the quaternary ammonium salt, and conjugated to gold nanoparticle scaffold to give the new SDC-1721-NP (Fig. 1l). This latter compound showed HIV-1 inhibition. Whereas SDC-1721 on its own did not inhibit viral replication, SDC-1721)-NP did with an IC_{50} of 10 nM. More important was the fact that at 48 h, viral production was insignificant when the TZM-b1 cells luciferase reporter gene system was used (Wei et al. 2003) to determine infectivity. This indicates that inhibition of viral replication was at the stage of viral entry (Bowman et al. 2008). Although the gold particles on their own had no effect on viral activity, they evidently played a role in the inhibitory activity of the modified SDC-1721 compound (SDC-1721)-NP). Metal gold in the form of nanoparticles therefore converted an inactive compound to an active form further indicating the potential of this metal in HIV therapy. Although this compound is unlike the mainstream molecular compounds such as those mentioned above, it is interesting to see how the particular gold-based nanoparticle conferred inhibition to HIV.

Discussion

In this review, different gold compounds demonstrating anti-HIV activity have been discussed with a focus on the type of inhibition and possible mechanisms of action. The majority of gold compounds that have anti-HIV action appear to exert their effect by inhibiting RT (Blough et al. 1989; Okada et al. 1993; Tepperman et al. 1994; Sun et al. 2004; Fonteh et al. 2009; Fonteh

and Meyer 2009). These kinds of inhibition by gold compounds are seemingly through ligand exchange reactions with sulfhydryl groups on the enzyme (Allaudeen et al. 1985). The reactive intermediates of the aurothiolates (bisAuTG and bisAuTM) inhibit the virus through the interaction of gold(I) with cysteinyl residues on the surface protein of the viral envelope as well as by direct enzyme inhibition of RT in cell free supernatant (Okada et al. 1993). The main mechanism of action of gold-based compounds therefore involves ligand exchange processes where the gold binds to thiol groups (of cysteine) on either RT or PR enzymes or any other protein (e.g. surface proteins on the viral envelope), thus altering the normal biological function of the protein. Auranofin, unlike AuTG and AuTM (which are injectable), is administered orally (Shapiro and Masci 1996) and demonstrated restoration of the immune system in an HIV patient being treated for psoriatic arthritis (Shapiro and Masci 1996). Even though there was no follow-up study involving auranofin, the initial study gives a pointer that this class of compounds should be interrogated closely. Another earlier report on two HIV infected patients with Reiter's syndrome and one with psoriatic arthritis also points to a good response to gold therapy (Espinoza et al. 1989). Dicyanogold(I), a common metabolite in patients on chrysotherapy has been shown to inhibit RT in vitro. According to Shaw (1999) conversion of gold compounds to dicyanogold(I) in patients on chrysotherapy make these compounds prodrugs i.e. the compounds are altered in vivo to form the active species.

General observations were that the inhibitory property of gold compounds was lost when the gold precursors i.e. $HAuCl_3$, $HAuCl_4.H_2O$ (Traber et al. 1999; Fonteh et al. 2009) and $KAu^{III}Cl_4$ (Sun et al. 2004) were tested for inhibition. Similarly, no significant inhibition was observed when the ligands alone such as TG or thiomalate (Okada et al. 1993; Traber et al. 1999) were tested. Phosphine ligands tested for anti-RT activity in our recent study (Fonteh and Meyer 2009) also did not show appreciable inhibition of the enzyme. The observed inhibitory effect of the gold compounds was therefore as a result of the complex and not the gold metal or ligand alone. Neither metallic gold nor ligands had any appreciable inhibition on their own. The concept of metal–ligand synergism (Navarro 2009) therefore enhances activity as shown for the gold compounds.

This review also highlights the fact that not only gold(I) compounds demonstrate inhibitory activity against HIV enzymes or pathogen entry but that gold(III) compounds with porphyrin ligands and other appropriate ligands could lead to inhibition of these enzymes or processes. Furthermore, modification of a therapeutically inactive monovalent small inorganic molecule to a highly active anti-HIV agent was observed by conjugation with gold nanoparticles.

Also worthy of note is the fact that inhibition in direct enzyme assays will not always give a complete picture of what could be expected in cell-based assays. For instance, where a compound could be directly active against an enzyme cell free, this activity may be lost in cell-based assays. This is because the compound might not cross the cell membrane to gain access to cell-based pathogens and their proteins. This kind of shortcoming can eventually influence in vivo applications.

Another consideration is that stability of gold compounds may be compromised after long term storage which could lead to altered activity. The gold(III) compound $[bpzmH_2][AuCl_4][Cl]$ (Fonteh et al. 2009) was tested not long after it was synthesized. Multiple repeats demonstrated its inhibition of RT over a concentration range of 5–250 μM. After a year and a half of storage at room temperature (in powder form), a retest demonstrated a loss in the RT inhibitory property. Sun et al. (2004) previously demonstrated that gold(III) porphyrins are stable in physiological medium (Liu et al. 1995; Che et al. 2003) and undergo no significant decomposition in the presence of excess glutathione. Similar stability profiles were seen for their work on $Au^{III}(TMPyP)Cl_5$ (Sun et al. 2004) where the compound remained stable in Tris buffer at room temperature. The stabilities were determined by UV/Vis spectroscopy. They also commented on the fact that no significant changes in the UV/Vis and ^1H NMR spectral data, acquired for $Au^{III}(TMPyP)Cl_5$ in the presence of excess GSH, over a period of time (Sun et al. 2004). However, none of the reports commented on the effect of long term storage, at room temperature, on the stability of the compounds. We are currently investigating the activity of $[bpzmH_2][AuCl_4][Cl]$ (Fonteh et al. 2009) under different storage conditions to ascertain the effect of temperature and long term storage on the powder and solution forms of the compound. This may also be a point of consideration

for other gold-based compounds and for researchers intending to pursue work related to gold compounds.

Overall it is clear that gold-compounds have therapeutic value that can be explored in HIV research. More importantly is that judicious choice of ligands appears to be the key to isolating stable gold(I) and gold(III) compounds which would presumably be less toxic and be more effective inhibitors.

References

Ahmad S (2004) The chemistry of cyano complexes of gold (I) with emphasis on ligand scrambling reactions. Coord Chem Rev 248:231–243

Allaudeen HS, Snyder RM, Whitman MH, Crooke ST (1985) Effects of coordinated gold compounds on in vitro and in situ DNA replication. Biochem Pharmacol 34:3243–3250

Angela C, Christian H, Chiara G, Enrico M, Paul JD, Bernard KK, Luigi M (2008) Gold(III) compounds as anticancer agents: Relevance of gold–protein interactions for their mechanism of action. J Inorg Biochem 102:564–575 and references therein

Argyris EG, Vanderkooi JM, Venkateswaran PS, Kay BK, Paterson Y (1999) The connection domain is implicated in metalloporphyrin binding and inhibition of HIV reverse transcriptase. J Biol Chem 274:1549–1556

Baba M, Nishimura O, Kanzaki N, Okamoto M, Sawada H, Iizawa Y, Shiraishim M, Aramaki Y, Okonogi K, Ogawa Y, Meguro K, Fujino M (1999) A small-molecule, non-peptide CCR5 antagonist with highly potent and selective anti-HIV-1 activity. Proc Natl Acad Sci USA 96:5698–5703

Best SL, Sadler PJ (1996) Gold drugs: mechanism of action and toxicity. Gold Bulletin 29:87–93

Blough H, Ricchetti M, Montagnier L, Buc H (1989) Organic gold compounds are effective against HIV-1 reverse transcriptase, vol 5. International conference on AIDS, Jun 4–9, p 559

Bowman M, Ballard TE, Ackerson CJ, Feldheim DL, Margolis DM, Melander C (2008) Inhibition of HIV fusion with multivalent gold nanoparticles. J Am Chem Soc 130:6896–6897

Caruthers SD, Wickline SA, Lanza GM (2007) Nanotechnological applications in medicine. Curr Opin Biotechnol 18:26–30

Che C-M (2004) Methods for using gold (III) complexes as anti-tumor and anti-HIV agents United States, patent: 2004, Pub No WO/2004/024146

Che C-M, Sun RW-Y, Yu W-Y, Ko C-B, Zhu N, Sun H (2003) Gold(III) porphyrins as a new class of anticancer drugs: cytotoxicity, DNA binding and induction of apoptosis in human cervix epitheloid cancer cells. Chem Commun (14):1718–1719

Crilly A, Madhok R, Watson J, Capell HA, Sturrock RD (1994) Production of interleukin 6 by monocytes isolated from rheumatoid arthritis patients receiving second line drug therapy. Br J Rheumatol 33:821–825

Espinoza LR, Aguilar JL, Berman A, Gutierrez F, Vasey FB, Germain BF (1989) Rheumatic manifestations associated with human immunodeficiency virus infection. Arthritis Rhuem 32:1615–1622

Fonteh P, Meyer D (2009) Novel gold(I) phosphine compounds inhibit HIV-1 enzymes Metallomics 1:427–433

Fonteh PN, Keter FK, Guzei I, Darkwa J, Meyer D (2009) Tetrachloro-(bis-3, 5-dimethylpyraxol)methane)gold(III)chloride: an HIV-1 reverse transcriptase and protease inhibitor. J Inorg Biochem 103:190–194

Fricker SP (1996a) Medical uses of gold compounds: past, present and future. Gold Bulletin 29(2):53–59

Fricker SP (1996b) Medicinal chemistry and pharmacology of gold compounds. Trans Metal Chem 21:377–383

Gibbs EJ, Maurer MC, Zhang JH, Reiff WM, Hill DT, Malicka-Blaszkiewicz M, McKinnie ER, Liu H-Q, Pasternack RF (1988) Interactions of porphyrins with DNA and more highly organised structures. J Inorg Biochem 32:39–65

Huaizhi Z, Yuantao N (2001) China's ancient gold drugs. Gold Bulletin 34(1):24–29

Keter FK, Ojwach SO, Oyetunji OA, Guzei IA, Darkwa J (2008) Bis(pyrazolyl)palladium(II), platinum(II) and gold(III) complexes: synthesis, molecular structures and reaction kinetics with L-Cysteine. Inorg Chim Acta 362: 2595–2602

Kovacs JA, Baseler M, Dewar RJ, Vogel S, Davey RT, Falloon J, Polis MA, Walker RE, Stevens R, Salzman NP, Metcalf JA, Masur H, Lane HC (1995) Controlled trial of interleukin-2 infusions in patients infected with the human immunodeficiency virus. N Eng J Med 335:1350–1356

Leibfarth JH, Persellin RH (1981) Mechanism of action of gold. Agents and Actions 11:458–472

Liu H-Q, Cheung T-C, Peng S-M, Che, C-M (1995) Novel luminescent cyclometalated and terpyridine gold(III) complexes and DNA binding studies. J Chem Soc Chem Commun 1787–1788

Loetscher P, Dewald B, Baggiolini M, Seitz M (1994) Monocyte chemottractant protein 1 and interleukin 8 production by rheumatoid synoviocytes. Effects of anti-rheumatic drugs. Cytokine 6:162–170

Madhok R, Crilly A, Murphy E, Smith J, Watson J, Capell HA (1993) Gold therapy lowers serum interleukin levels in rheumatoid arthritis. J Rheumatol 20:630–633

Mascarenhas B, Granda J, Freyberg R (1972) Gold metabolism in patients with rheumatoid arthritis treated with gold compounds-reinvestigated. J Rheumatol 23:1818–1820

Miller S (2002) Beyond the CD4 count. The clinician's guide to the immunity of HIV infection. Beckman Coulter 3(7):19–23, 27

Navarro M (2009) Gold complexes as potential anti-parasitic agents. Coord Chem Rev 253:1619–1625

Navarro M, Perez H, Sanchez-Delgado GA, Schmidbaur H (1997) Toward a novel metal-based chemotherapy against tropical diseases. 3. Synthesis and Antimalarial Activity in vitro and in vivo of the New Gold-Chloroquine Complex $(Au(PPh_3)(CQ))PF_6$. J Med Chem 40:1937–1939

Okada T, Patterson BK, Ye S, Gurney ME (1993) Aurothiolates inhibit HIV-1 infectivity by gold (I) ligand exchange

with a component of the virion surface. Virology 192:631–642

Pallela FJ, Delaney KM, Moorman AC, Loveless MM, Fuhrer J, Satten GA, Aschman DJ, Holmberg SD (1998) Declining morbidity and mortality among patients with advance human Immunodeficiency virus infection. HIV outpatient study investigations. N Engl J Med 338:853–860

Parish RV, Cottrill SM (1987) Medicinal gold compounds. Gold bulletin 20(1/2):3–11

Sadler PJ, Guo Z (1998) Metal complexes in medicine: design and mechanism of action. Pure Appl Chem 70(4):863–871

Sadler PJ, Li H, Sun H (1999) Coordination chemistry of metals in medicine: target sites for bismuth. Coord Chem Rev 185–186:689–709

Safarian D, Carnec X, Tsamis F, Kajumo F, Dragic T (2006) An anti-CCR5 monoclonal antibody and small molecule CCR5 antagonists synergize by inhibiting different stages of human immunodeficiency virus type 1 entry. Virology 352:477–484

Saqqioro D, Rigobello MP, Paloschi L, Folda A, Moqqach SA, Parsons S, Ronconi L, Fregona D, Bindoli A (2007) Gold(III)-dithiocarbamato complexes induce cancer cell death triggered by thioredoxin redox system inhibition and activation of ERK pathway. Chem Biol 14:1128–1139 (references therein)

Seitz M, Dewald B, Ceska M, Gerber N, Baggiolini M (1992) Interleukin-8 in inflammatory rheumatic diseases : synovial fluid levels, relation to rheumatoid factors, production by mononuclear cells, and effects of gold sodium thiomalate and methotrexate. Rheumatol Int 12:159–164

Shapiro DL, Masci JR (1996) Treatment of HIV associated psoriatic arthritis with oral gold. J Rheumatol 23:1818–1820

Shaw CF III (1979) The mammalian biochemistry of gold: an inorganic perspective of chrysotherapy. Inorg Perspect Biol Med 2:287–355

Shaw CF III (1999) Gold-based therapeutic agents. Chem Rev 99:2589–2600

Stern I, Wataha JC, Lewis JB, Messer RLW, Lockwood PE, Tseng WY (2005) Anti-rheumatic gold compounds as sublethal modulators of monocytic LPS-induced cytokine secretion. Toxicol In Vitro 19:365–371

Suerbaum S, Michetti PN (2002) Helicobacter pylori infection. N Engl J Med 347:1175–1186

Sun RW-Y, Sun H, Che C-M (2004) In vitro inhibition of human immunodeficiency virus type-1 (HIV-1) reverse transcriptase by gold(III) porphyrins. Chem Bio Chem 5:1293–1298

Sun RW-Y, Ma D-L, Wong EL-M, Che C-M (2007) Some uses of transition metal complexes as anti-cancer and anti-HIV agents. Dalton Trans 43:4884–4892

Sutton BM (1986) Gold compounds for rheumatoid arthritis. Gold Bulletin 19(1):15–16

Tepperman K, Zhang Y, Roy PW, Floyd R, Zhao Z, Dorsey JG, Elder RC (1994) Transport of the diacyanogold (I) anion. Met Based Drugs 1(5–6):433–443

Traber KE, Okamoto H, Kurono C, Baba M, Saliou C, Soji T, Parker L, Okamoto T (1999) Anti-rheumatoid compound aurothioglucose inhibits tumour necrosis-α-induced HIV-1 replication in latently infected OM10.1 and Ach2 cells. Int Immunol 1(2):143–150

Wanchu A (2003) HIV infection: rheumatic manifestation. J Indian Rheumatol Assoc 11:121–130

Wei X, Decker JM, Wang S, Hui H, Kappes JC, Xiaoyun WX, Salaza-Gonzalez JF, Salazar MG, Kilby JM, Saag MS, Komarova NL, Nowak MA, Hahn BH, Kwong PD, Shaw GM (2003) Antibody neutralization and escaby by HIV-1. Nature 422:307–312

Yamaguchi K, Ushijima H, Hisano M, Inoue Y, Shimamura T, Hirano T, Muller WEG (2001) Immunodeficiency effect of gold sodium thiomalate on murine acquired immunodeficiency syndrome. Microbiol Immunol 45:549–555

Yang JP, Merin JP, Nakano T, Kato T, Kitade S, Okamoto T (1995) Inhibition of the DNA-binding activity of NF-kappa B by gold compounds in vitro. FEBS Letters 361:89–96

Yanni G, Nabil M, Farahat MR, Poston RN, Panayi GS (1994) Intramuscular gold decreases cytokine expression and macrophage numbers in the rehumatoid synovial membrane. Ann Rheum Dis 53:315–322

Yoshida S, Kato T, Sakurada S, Kurono C, Yang JP, Matsui N, Soji T, Okamoto T (1998) Inhibition of IL-6 and IL-8 induction from cultured rheumatoid synovial fibroblasts by treatment with aurothioglucose. Int Immunol 11:151–158

Zhang Y, Hess EV, Pryhuber KG, Dorsey JG, Tepperman K, Elder RC (1995) Gold binding sites in red blood cells. Inorg Chim Acta 229:271–280