Gold Compounds as Anticancer Agents: Chemistry, Cellular Pharmacology, and Preclinical Studies

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Abstract: Gold compounds are a class of metallodrugs with great potential for cancer treatment. During the last two decades, a large variety of gold(I) and gold(III) compounds are reported to possess relevant antiproliferative properties in vitro against selected human tumor cell lines, qualifying themselves as excellent candidates for further pharmacological evaluation. The unique chemical properties of the gold center confer very interesting and innovative pharmacological profiles to gold-based metallodrugs. The primary goal of this review is to define the state of the art of preclinical studies on anticancer gold compounds, carried out either in vitro or in vivo. The available investigations of anticancer gold compounds are analyzed in detail, and particular attention is devoted to underlying molecular mechanisms. Notably, a few biophysical studies reveal that the interactions of cytotoxic gold compounds with DNA are generally far weaker than those of platinum drugs, implying the occurrence of a substantially different mode of action. A variety of alternative mechanisms were thus proposed, of which those involving either direct mitochondrial damage or proteasome inhibition or modulation of specific kinases are now highly credited. The overall perspectives on the development of gold compounds as effective anticancer drugs with an innovative mechanism of action are critically discussed on the basis of the available experimental evidence. Med Res Rev, 30, No. 3, 550–580, 2010

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1. INTRODUCTION

Gold compounds have a long and important tradition in medicine (also known as *chrysotherapy*), which dates back to the ancient Egyptians and especially flourished during the late Middle Age and the Renaissance. Gold compounds were widely used in early times of modern pharmacology, for the treatment of several diseases, especially as anti-infective and antitubercular agents. Despite extensive clinical experimentation carried out at that pioneering time, gold compounds have had limited medical application and are presently used only for the treatment of severe rheumatoid arthritis.[1,2] This is probably the result of their relevant systemic toxicity (e.g. nephrotoxicity) and of the poor chemical stability of the tested compounds. However, we believe that the unique chemistry of the gold center is not yet fully appreciated and may be further used for different and more relevant pharmacological purposes, especially in the field of anticancer medicine.

Following the clinical use of cisplatin, one of the first metal complexes to be extensively employed for cancer treatment,[3] there have been several attempts to prepare and evaluate new gold compounds—either gold(III) or gold(I)—as experimental antitumor agents with particular focus on gold phosphine complexes.[4,5] Some of these compounds showed very pronounced and encouraging antitumor actions in vitro and were the object of great attention; however, they were rapidly abandoned due to the occurrence of severe toxicity and to the generally lower in vivo efficacy (in comparison with the in vitro effects). In recent years, many new gold compounds—in most cases gold(III) compounds—have been synthesized and characterized, which show greater chemical stability and a far better toxicological profile, which undoubtedly warrant additional pharmacological testing. The structural and solution chemistry of these novel gold compounds and their reactivity with a variety of possible biomolecular targets are the object of recent reviews and will be briefly outlined here.[6,7]

The main scope of this article is to describe the most representative pharmacological studies on anticancer gold compounds. Although this topic is essential to the development of new drug-lead compounds as well as for the planning of future clinical studies, it has been very marginally considered in the previous literature and deserves, in our opinion, far greater attention.

2. CHEMISTRY OF GOLD COMPOUNDS

A. General Remarks

The chemistry of gold contains some unique aspects that are most likely the consequence of important unique electronic properties of the gold center. For instance, gold has a great propensity to form strong gold–gold bonds (the so-called "aurophilic interactions").[8] A rich redox chemistry is associated with the three main oxidation states of gold. In turn, redox changes are strictly linked to changes in the coordination sphere with a frequent switch from square planar gold(III) complexes to linear dicoordinated gold(I) complexes.[2] All these aspects may be exploited to build up a variety of gold-based pharmacologically active compounds.

The most important oxidation states for gold are the following: Au(0), Au(I), and Au(III). The elemental forms of gold, principally metallic and colloidal gold, are stable; however, in the presence of relatively strong ligands, elemental gold can undergo rather facile oxidation. The chemistry of gold complexes in the oxidation states +1 and+3 has been investigated in depth as well as their behavior in solution and reactivity with

biomolecules.[2,9,10] A relatively easy interchange between the oxidation states +1 and+3 even under physiological conditions is possible.[4] This specific property may be pharmacologically relevant. There are some excellent books and reviews[2,11] for readers wanting a more extensive description of gold chemistry.

B. Gold(I) Compounds

Gold(I) has a d^{10} closed-shell configuration, which is the basis for three principal coordination environments: linear two-coordination, by far the most important, but also trigonal three-coordination and tetrahedral four-coordination.

Gold(I) is a soft cation with a preference for soft ligands. Thus, cyanide, thiolate, and soft halides (e.g. iodide) tend to form stable AuX_2^- ions; on the other hand, phosphines, arsines, and other neutral ligands readily form a variety of cationic complexes of the AuL_2^+ and AuL_4^+ type.

The most important gold(I) compounds for medicinal purposes are thiolate and phosphine complexes, which are most often dicoordinate gold(I) complexes. Some representative examples of medicinally relevant gold(I) compounds, notably, auranofin, aurothiomalate, aurothioglucose, and auro-*bis*(thiosulfate), are shown in Figure 1. Structural data are available for the reported compounds.[1] Auranofin exists as a monomeric species, while the other compounds have been isolated in solid state in oligomeric forms. The aqueous solution chemistry of these compounds has been intensely investigated[10,12] and indicates that thiolate ligands are usually more labile than phosphine ligands and undergo rapid aquation. The resulting cationic species usually show a strong reactivity with biomolecules. Analogously, phosphine ligands may undergo aquation but these reactions are generally far slower.

The electrochemical behavior of gold(I) drugs has also been investigated in detail.[13,14] Auranofin can undergo reduction to colloidal gold, the process being irreversible. Cyclic voltammetry studies show sufficiently high reduction potentials for auranofin, myocrisin, and the parent compounds so that all these compounds may be easily and quickly reduced by glutathione and other thiols within the cellular environment.

C. Gold(III) Compounds

Gold(III) has a d^8 closed-shell configuration, which originates metal complexes that are isoelectronic and isostructural to platinum(II) complexes; accordingly, the dominant coordination geometry for gold(III) complexes is square planar tetra-coordination. The bond lengths are shorter than the corresponding gold(I) bond lengths although differences are not

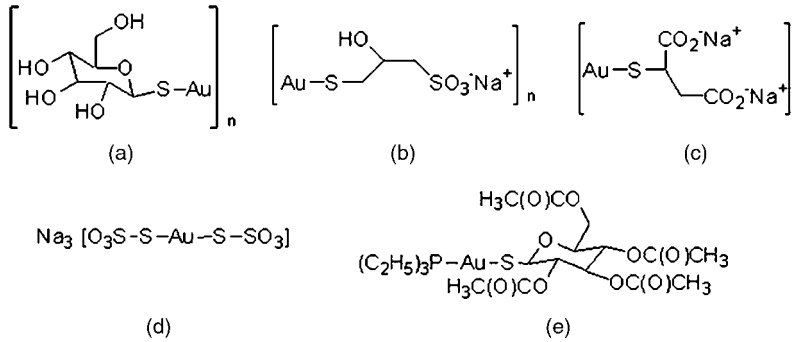

Figure 1. Schematic drawing of some gold(I) thiolates used in the treatment of rheumatoid arthritis: aurothioglucose (solganol) (**a**), aurothiopropanol sulfonate (allocrysin) (**b**), aurothiomalate (myochrysine) (**c**), auro-*bis*(thiosulfate) (sanochrysine) (**d**), and auranofin (ridaura) (**e**).

very large. Five- and six-coordinate complexes are also found that typically exhibit elongated axial bond lengths perpendicular to the square plane and often involve ligand structures that constrain the axial donor atoms. The reactivity behavior of the gold(III) cation is borderline, showing a preference not only for soft ligands but also for nitrogen donors. The oxidation state +3 is strongly oxidizing unless the gold(III) cation is stabilized by an appropriate set of nitrogen or soft donors.

A vast array of gold(III) complexes showing rather variegated structural chemistry have been considered as potential anticancer drugs[9] and several of them are discussed in this review. The gold(III) compounds of interest may be grouped into the four following classes: (i) classical mononuclear gold(III) complexes; (ii) gold(III) porphyrins; (iii) organogold(III) compounds; (iv) dinuclear gold(III) complexes.

1. Classical Mononuclear Gold(III) Complexes

The main compounds of this class are shown in Figure 2. They are square planar gold(III) compounds mostly with nitrogen or halide ligands: $AuCl_4^-$, $[Au(dien)Cl]Cl_2$ (Audien), $[Au(en)_2]Cl_3$ (Auen), $[Au(cyclam)](ClO_4)_2Cl$ (Aucyclam), $[Au(terpy)Cl]Cl_2$ (Auterpy), $[Au(phen)Cl_2]Cl$ (Auphen).[15] Structural data are reported for all these compounds. As nitrogen ligands are stronger and less labile than chloride ligands, it follows that chloride ligands undergo far more facile aquation reactions that lead to complex activation. In turn, nitrogen ligands induce significant stabilization of the oxidation state +3. The solution behavior of these compounds has been investigated. $AuCl_4^-$ is poorly stable in physiological pH due to rapid and progressive release of its chloride ligands; in turn, Aucyclam is very stable due to the equatorial donor set. In the other cases chloride release has been monitored affording cationic species with at least one reactive coordination position. Notably, other compounds belonging to this group are gold(III) dithiocarbamate complexes. The compounds containing *N*,*N*-dimethyldithiocarbamate and ethylsarcosinedithiocarbamate ligands, developed in Padua, have been intensely studied as possible anticancer agents (Fig. 3, compounds 5 and 6).[16]

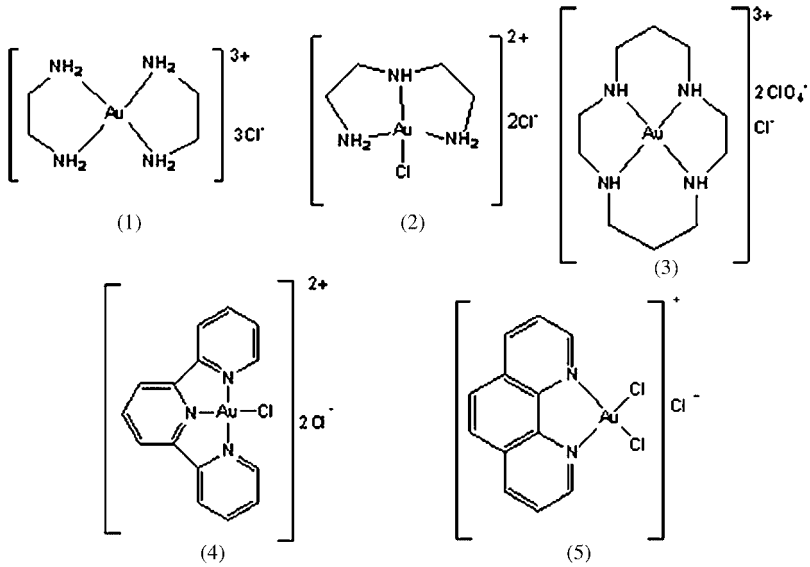

Figure 2. Schematic drawing of $[Au(en_2)]Cl_3$ (**1**), $[Au(dien)Cl]Cl_2$ (**2**), $[Au(cyclam)](ClO_4)_2Cl$ (**3**), $[Au(terpy)Cl]Cl_2$ (**4**), and $[Au(phen)Cl_2]Cl$ (**5**).

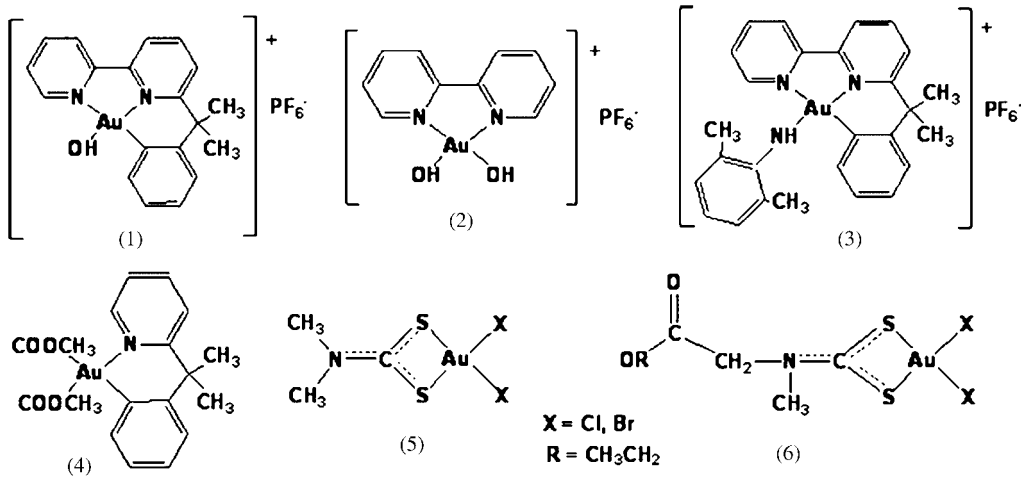

Figure 3. Schematic drawing of [Au(bipy^{c}-H)(OH)][PF_6] (**1**), [Au(bipy)$(OH)_2$][PF_6] (**2**), [Au(bipy^{dmb}-H)(2,6-xylidine-H)][PF_6] (**3**), [Au(py^{dmb}-H)$(AcO)_2$] (**4**) (where py^{dmb} = 2-(1,1-dimethylbenzyl)-pyridine), and of the gold(III) dithiocarbamate complexes containing *N,N*-dimethyldithiocarbamate (**5**) and ethylsarcosinedithiocarbamate (**6**) ligands.

2. Gold(III) Porphyrins

The porphyrin ligand greatly stabilizes the gold(III) center and drastically reduces its redox reactivity and oxidizing character.[17] Crystal structures have been reported.[17] It is very unlikely that metalloporphyrin demetallation may occur with the release of the gold(III) ion. Similarly gold(III) reduction to gold(I) or elemental gold is very unlikely. This means that the biological activity of gold(III) porphyrins must be ascribed to the intact molecule. It has been hypothesized that the primary target for gold(III) porphyrins is DNA following intercalation; however, recent studies reveal that gold(III) porphyrins may greatly affect mitochondrial functions as well.[18]

3. Organogold(III) Compounds

Gold(III) compounds belonging to this class are characterized by the presence of at least one direct carbon–gold(III) bond; this latter feature is very important for the stabilization of gold oxidation state +3. Examples of organogold(III) compounds relevant to this review are shown in Figure 3. Organogold(III) compounds are generally stable under physiological conditions and have a scarce tendency to be reduced to gold(I). They are significantly cytotoxic to human tumor cell lines.[19,20]

4. Dinuclear Gold(III) Complexes

A series of structurally related oxo-bridged dinuclear gold(III) compounds, [$Au_2(\mu\text{-}O)_2$ $(N\hat{}N)_2$][$PF_6)_2$, where $N\hat{}N$ is 2,2′-bipyridine or a substituted 2,2′-bipyridine, have recently been shown to exhibit appreciable stability under physiological-like conditions and to manifest important antiproliferative effects toward selected human tumor cell lines (Fig. 4).

All these compounds contain a common structural motif consisting of an Au_2O_2 "diamond core" linked to two bipyridine ligands in a roughly planar arrangement. Interestingly, the introduction of different kinds of alkyl or aryl substituents on the 6 (and 6′) position(s) of the bipyridine ligand leads to small structural changes that nonetheless greatly affect the reactivity of the metal centers. The 6,6′-dimethyl-2,2′-bipyridine derivative, which shows the largest structural deviation compared with the model compound [$Au_2(\mu\text{-}O)_2(bipy)_2$][$PF_6)_2$, also has the highest oxidizing power, the least thermal stability, and the greatest cytotoxic

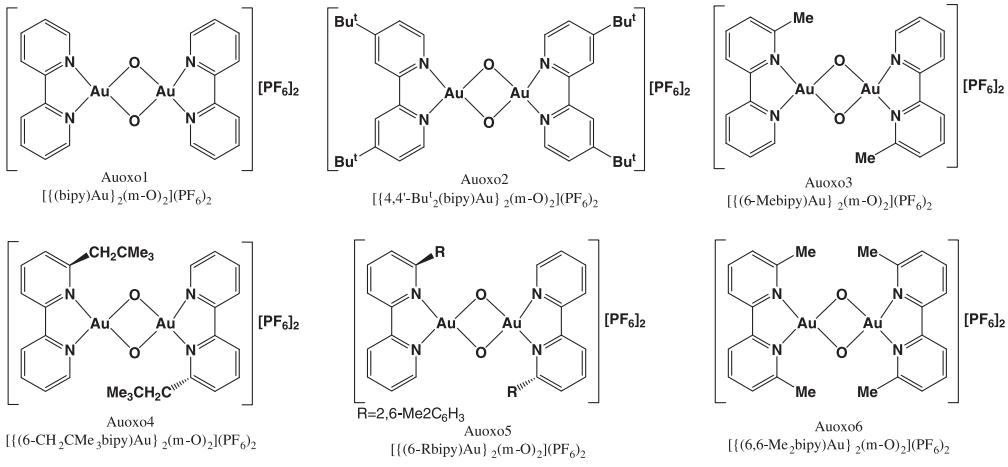

Figure 4. Schematic drawings of the dinuclear gold(III) complexes Auoxo. Auoxo3 is a ca. 1:1 mixture of the *cis*- and *trans*-isomer, while Auoxo4 and Auoxo5 are, as depicted, only *trans*-isomers.

activity. The positive correlation between the oxidizing power and the antiproliferative effects seems to be of particular interest.[8]

3. TRANSPORT, BIODISTRIBUTION, AND BIOTRANSFORMATION OF GOLD COMPOUNDS

A variety of chemical, biochemical, and biological studies (both in animals and in patients) indicate that gold(I) compounds—in clinical use for as long as antiarthritic agents—are *prodrugs* as they must undergo specific chemical transformations to generate their pharmacologically active species.[2,21] These chemical transformations usually consist of fast ligand exchange reactions. A number of important gold metabolites may be produced as well. Thus, $[Au(CN)_2]^-$ (aurocyanide), gold–glutathione complexes, and gold–protein adducts seem to play major roles in the overall in vivo metabolism of gold compounds.[2] Detailed information is available on the metabolism, transport, and biodistribution of gold(I) complexes in relation to several studies carried out so far on antiarthritic gold(I) drugs. Details will be described below. Conversely, only limited data are available regarding the metabolism of experimental gold(III) compounds and their intracellular disposition and fate. In any case, various bioinorganic studies performed on gold(III) metallodrugs in recent years point out that gold(III) compounds exhibit, on the whole, relevant reactivity with several biomolecules consisting of either ligand exchange processes or redox processes. Depending on the specific nature of the various gold(III) complexes, their electrochemical profile, and the type of reacting species, these reactions may lead either to the formation of tight gold(III)–biomolecule coordinative bonds or to the oxidation and damage of the involved biomolecule itself.[9]

Due to the high affinity of the ''soft'' gold(I) center for sulfur and selenium ligands, proteins (e.g. enzymes, transport proteins) bearing accessible side chains like cysteine, methionine, and selenocysteine constitute preferential targets for gold(I) compounds.[1] However, gold(I) coordination to imidazole groups of histidines is also possible. Commonly, reactions of gold drugs or their metabolites with proteins result in the formation of tight gold–protein adducts so that gold is usually associated with high-molecular-weight components.[9]

Gold(I) thiolate drugs as well as gold(I) phosphines have been reported to undergo important ligand exchange reactions with the tripeptide glutathione or with proteins like

albumin and metallothioneins (MTs).[22–24] Gold(I) is thermodynamically more stable than gold(III). However, in the lysosomes of activated macrophages and granulocytes, i.e. one of the main intracellular storage sites for gold compounds, injected gold(I) drugs may be oxidized to gold(III) by hypochlorite (generated by myeloperoxidase).[25,26] This represents an effective and unexpected biochemical pathway whereby the usually unstable gold(III) species are formed in vivo.

Much attention has been focused on the study of the reactions of gold(I) compounds with serum albumin, a protein abundant in the blood, which can transport metal compounds (Fig. 5). Among the several cysteine residues present in the serum albumin, the low pK_a cysteine-34, which is predominantly deprotonated at the physiological pH, seems to be the most likely anchoring site for gold drugs in the blood. The coordination of aurothiomalate to cysteine-34 to form albumin-S-Au-STm and albumin-S-(Au-μSTm)$_n$-Au-STm is supported by chemical evidence.[27]

Much is known about the reaction of auranofin with serum albumin. A ligand exchange reaction of auranofin with cysteine-34 displaces its original thiol ligand—i.e. the tetraacetylthioglucose ligand.[27–31] In addition, a conformational change in albumin, which accompanies gold binding to cysteine-34, has been described.[32] In turn, the tetraacetylthioglucose ligand released from auranofin may react further with cysteine-34 disulfide bonds to liberate cysteine[33]; the triethylphosphine (Et_3P) ligand, once released from auranofin, is rapidly oxidized to triethylphosphine oxide (Et_3PO).[34]

Physiological low-molecular-weight thiol ligands like glutathione may induce the displacement of phosphine[34,35] and its oxidation. The oxidant for phosphine can be either molecular oxygen or albumin disulfide bonds.[36] As free oxygen is not present in the serum, it is likely that disulfide bonds are the actual in vivo oxidants.[36]

The three components of auranofin are thus metabolized very differently in vivo: triethylphosphine oxide and the thiol ligand are excreted with half-lives of 8 and 16 hr, respectively, while the half-life for gold excretion is about 20 days.[37] When auranofin is added to whole blood, ligand displacement reactions are extremely rapid. Within 20 min, gold is primarily protein bound in the serum, while Et_3P is distributed in 1:2:2 ratio among serum proteins, red cells, and Et_3PO,[38] which is then excreted by the kidneys.[39]

In animal models, gold(I) compounds are known to bind strongly and extensively to MT, a heavy-metal binding protein that is abundant in mammalian kidney and liver[40] (Fig. 5). Under normal physiological conditions, MT can bind seven Zn(II) or Cd(II) ions in the two metal clusters generated from its 20 cysteine residues. MT is found to manifest a high affinity for gold(I) and to displace the thiomalate ligand from AuSTm; the resulting protein-bound gold is coordinated with two cysteine residues to form Au,Cd-MT or Au,Cd,Zn-MT clusters, depending on the initial metal loading of MT.[41] In view of these interesting results it seems very likely that MT plays a crucial role in the intracellular gold storage and metabolism.

Gold(I) drugs may also be activated through conversion to $Au(CN)_2^-$, a common metabolite for gold(I) compounds, which is normally recovered in the blood and urine of gold-treated patients.[2] $Au(CN)_2^-$ may be produced by two processes involving the formation of hypothiocyanide and hypochlorous acid. $Au(CN)_2^-$ is known to be an inhibitor of the respiratory burst of neutrophils and monocytes and of lymphocyte proliferation. The neutrophil enzyme myeloperoxidase may convert gold thiomalate to $Au(CN)_2^-$ through the oxidation of thiocyanate.[42]

As previously mentioned, gold(III) is apparently an intermediate metabolite formed in vivo through the oxidation of gold(I). The oxidation of gold(I) to gold(III) for sodium aurothiomalate seems to be responsible for the adverse immune reactions that may develop during gold therapy.[43] In vitro studies with phagolysosomes suggest the presence of a redox system and the formation of gold(III) from gold(I) following an oxidative burst in phagocytic

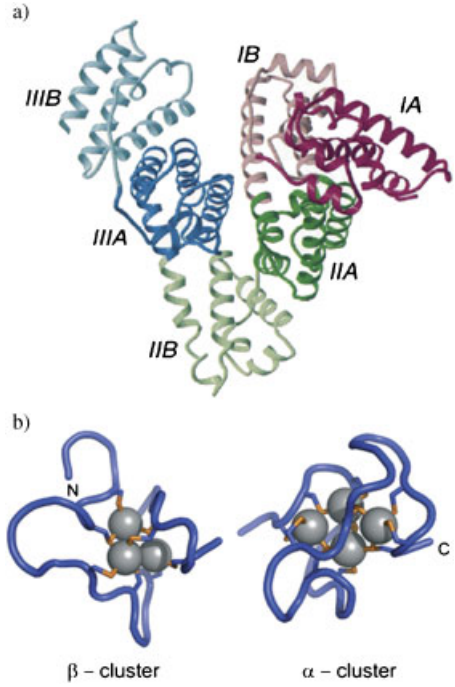

Figure 5. Molecular structures of (**a**) human serum albumin (reprinted from Curry et al., BBA---Mol Cell Biol Lipids 1999;1441:131–140. Copyright 1999, with permission from Elsevier) and (**b**) metallothionein-2 (from rat liver), two proteins that are primarily involved in gold transport and storage.

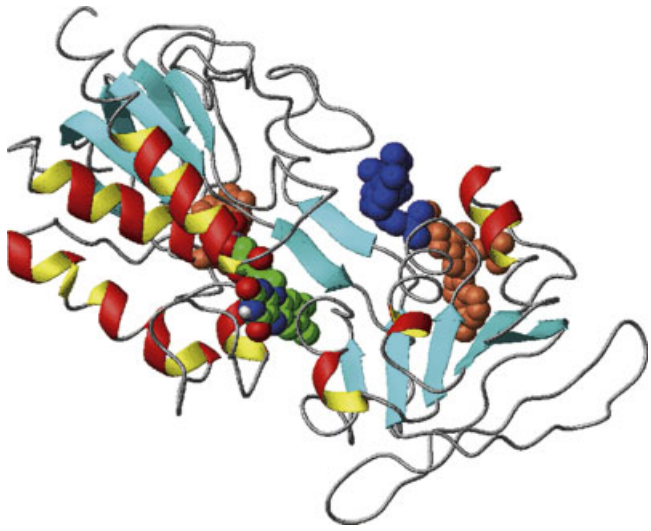

Figure 6. Schematic representation of the molecular structure of mammalian thioredoxin reductase TrxR1 (reprinted from Casini et al.[9]. Copyright 2008, with permission from Elsevier).

immune cells.[44] Conversely, the reduction of $AuCl_3$ and of its derivatives by serum albumin and by a variety of thiols and thioethers occurs over longer time periods compared with the hypochlorite oxidation of gold(I).[44]

Overall, the described results point out that gold(I) drugs have a rich and complex coordination chemistry inside cells and may undergo a variety of chemical transformations, mainly driven by the intracellular thiols. The interplay of gold between the oxidation states +1 and+3 has been conclusively demonstrated. Some studies have described the interactions of gold drugs with membranes. Due to the presence of solubilizing groups, gold(I) thiolates (including aurothiomalate and aurothioglucose) are water soluble compounds; therefore, they do not enter cells, but rather bind to the cell membrane via cell surface thiols. Consequently, these drugs may affect cell metabolism either by interfering with normal cell signalling pathways or by starving cells through inhibition of nutrient uptake.[22,45,46]

The mechanism of transport of auranofin metabolites (i.e. $Au(CN)_2^-$) has been investigated in depth by Snyder et al.[47] in cultured macrophage cells. They postulate the thiol-shuttle (or the sulfhydryl-shuttle) model for gold entry inside cells. Auranofin reaches the membrane of various cell types unmodified.[37,47,48] Sulfhydryl-dependent membrane transport proteins provide a vehicle for the movement of the Et_3PAu^+ cation across the cell membrane, whereas the thiolate ligand remains outside the cell. Then, the cation is transferred to proteins and to low-molecular-weight thiols while the phosphine is oxidized to Et_3PO. Either bound or unbound to phosphine, the gold ion can be shuttled out of the cell the same way as it enters it.[47,48] At this time, gold may still be bound to an intracellular thiol (albumin or glutathione). Once outside the cell, gold again binds to an extracellular thiol (e.g. albumin), resulting in the formation of a complex constituted by albumin, gold, and an intracellular thiol. This complex may represent the major circulating metabolite of auranofin. Membrane transport proteins that mediate gold uptake are not an energy-dependent active transport process.[47] Thus, the hypothesis of a possible equilibrium between intracellular gold concentrations and extracellular gold sources has been successfully investigated.[48]

4. CELLULAR AND MOLECULAR TARGETS OF GOLD COMPOUNDS

Due to the numerous in vitro studies that have appeared on gold compounds in the last decade, a number of specific hypotheses have been formulated concerning their possible mode of action. Nevertheless, the molecular mechanisms responsible for their biological effects are still largely unknown. The conspicuous amount of pharmacological data now available for a variety of gold compounds has led to the identification of a few crucial cellular processes, likely involved in their cytotoxic actions, which should be further explored. In particular, direct DNA damage, modification of the cell cycle, mitochondrial damage, proteasome inhibition, modulation of specific kinases, and other cellular processes affected by gold compounds, which eventually trigger apoptosis, seem to play a major role in the mechanism of action of gold compounds. The main postulated biomolecular targets and biochemical mechanisms that emerge from those investigations are reviewed below.

Overall, from a detailed analysis of the mechanistic studies carried out so far on the biological reactions of gold compounds, it is possible to define three major classes according to their mode of action with biological targets.

(1) Gold compounds are *prodrugs* capable of coordinating tightly to biomolecules' side chains, e.g. thiols, imidazole, and selenols, after activation (usually through the release of a labile ligand), e.g. auranofin. This behavior involves an alkylation mechanism similar to that of the platinum compounds.
(2) Gold complexes are big cations capable of crossing membranes and of binding noncovalently but strongly to biomolecules (proteins, enzyme, DNA) (coordination

compounds that target biomolecules) e.g. gold-based DNA intercalators, gold(III) porphyrins.

(3) Gold compounds that react with biomolecules mainly through redox chemistry causing oxidative damage e.g. Auoxo6.

A. DNA as a Target

Initially, mechanistic studies on gold compounds have focused on DNA and RNA as these biomolecules are commonly considered to be the major targets for anticancer platinum drugs. However, weak interactions occurring in vitro between antiarthritic gold(I) drugs and DNA in early studies were confirmed in later studies, disclosing the possibility that different cellular processes might be involved in the cytotoxic mechanism of gold(I) drugs.[49] In turn, the marked "soft" character of the gold(I) center makes a selective and tight reaction with the nitrogen donors of nucleobases rather unlikely; recent studies revealing strong interactions of gold(I) drugs with specific protein side chains such as thiols and selenols rather than with nucleobases indirectly support that early view.[5,50]

More extensive studies have been carried out on the reactions of gold(III) compounds with DNA upon consideration of the "harder" character of the gold(III) center and of its high propensity to react with nucleobase nitrogens. Significantly stronger interactions with DNA and DNA nucleobases are highlighted in comparison with gold(I) drugs. As gold in the +3 oxidation state is isoelectronic (d^8) with platinum(II) and forms square planar complexes, cisplatin is the obvious comparative drug for most of the mechanistic studies carried out on gold(III) complexes. However, the molecular mechanisms of gold(III) compounds and cisplatin are still quite different. Although it was suggested that possible binding sites for gold(III) compounds are the same as for platinum(II) drugs,[51,52] it still remains to be seen to what extent gold(III) compounds react with DNA and not with other intracellular targets (i.e. proteins or low-molecular-weight thiols).

We analyzed the interactions of a series of structurally different gold(III) compounds with DNA in detail using several physicochemical techniques. Our studies reveal that the in vitro interactions of mononuclear bipyridyl gold(III) complexes (such as $[Au(bipy)(OH)_2][PF_6]$ and $[Au(bipy^c\text{-}H)(OH)][PF_6]$),[53] of polyamine complexes (such as $[Au(en)_2]Cl_3$ and $[Au(dien\text{-}Cl]Cl_2$),[54] and of a series of dinuclear oxo gold(III) complexes bearing bipyridyl ligands[55] with *calf-thymus* DNA are usually weak and reversible, suggesting that they are mainly electrostatic in nature. However, there are some notable exceptions. $[Au_2(6,6'\text{-dimethyl-}2,2'\text{-bipyridine})(\mu\text{-}O)_2][PF_6]_2$ is reported to give rise to specific redox processes and to bind firmly to the DNA double helix.[55] Also, chloroglycylhistidine gold(III) compounds are reported to bind DNA tightly and modify appreciably its conformation.[56] The case of a gold(III) terpyridine (Terpy) complex, $[AuCl(Terpy)]Cl_2$, a gold(III) analogue of [PtCl(Terpy)]Cl, endowed with very relevant cytotoxic properties[15] merits more extensive analysis. This complex was investigated to establish whether it may act as an intercalating agent like [PtCl(Terpy)]. In spite of their close structural similarity, the comparative study of the interactions of both compounds with double-stranded DNA shows that they produce quite different DNA interaction patterns. It also emerges that the resulting metal/DNA interaction patterns depend on the applied incubation times. Remarkably, [PtCl(Terpy)]Cl quickly intercalates DNA; then, coordinative bonds form progressively over time. At variance, $[AuCl(Terpy)]Cl_2$ first interacts electrostatically with the DNA surface, with subsequent slow formation of some coordinative bonds.[57]

A recent study shows that the level of DNA metallation induced by two gold(III) terpyridine complexes is comparable to that of DNA platination by *trans*-platinum

complexes.[58] In addition, the same authors found that for complexes bearing a different positive charge, a higher positive charge enhances the DNA binding affinity. The main interactions of these complexes with DNA are ascribed to the intercalation of the square planar gold(III) chromophore into the DNA double helix.

DNA binding affinity studies performed using purified calf-thymus DNA on some gold(III) dithiocarbamate derivatives (i.e. $[Au(DMDT)X_2]$ where DMDT is *N*,*N*-dimethyldithiocarbamate and $[Au(ESDT)X_2]$ where ESDT is ethylsarcosinedithiocarbamate; X = Cl or Br) show that they possess an appreciable affinity for the DNA double helix with some evidence of binding being achieved soon after mixing.[59] Thus, the studied gold(III) dithiocarbamate derivatives appear to be more efficient in inducing interstrand cross-links than cisplatin itself. In particular, gold(III)–DNA adducts are formed with faster kinetics compared with cisplatin but turn out to be less stable.[59] Notably, the resulting DNA lesions are very efficient in killing cells.

There is much recent attention focused on the analysis of the interactions of cytotoxic gold(III) porphyrins with DNA. The first mechanistic studies on gold(III) porphyrins (using gold(III) mesotetraarylporphyrins) show that they interact strongly and directly with DNA,[17] implying that DNA may actually constitute a preferential biomolecular target. However, in a successive study, the same authors reveal that gold(III) porphyrin 1a acts differently from cisplatin in vivo as the gold compound causes DNA fragmentation rather than cross-linkage; moreover, its interactions with DNA are reported to be noncovalent and reversible in nature.[60]

It seems reasonable to conclude that DNA is neither the primary nor the exclusive target for most gold(I) and gold(III) complexes. A strong gold association with DNA has been demonstrated only in a few cases, in particular those where the interaction is mainly dependent on the nature of the ligand (as is the case for gold porphyrins and gold terpyridines). Accordingly, the relevant cytotoxic effects induced by gold compounds must logically arise from their ability to interfere with distinct cellular processes and targets.[61] A few relevant targets for gold compounds, mainly protein targets, have clearly emerged during the last years and are described below.

B. Proteins as Targets: Inhibition of Thioredoxin Reductase

The action of gold compounds on the enzyme thioredoxin reductase, now described in depth, might explain the relevant alterations observed in mitochondrial functions (e.g. permeability transition) after gold treatment.[62,63] The thioredoxin redox system comprises thioredoxin reductase (TrxR), a homodimeric selenium containing flavoprotein, and thioredoxin (Trx), a ubiquitously expressed small protein with a conserved Cys-Gly-Pro-Cys redox catalytic site capable of reducing a variety of substrates.[64] Both Trx and TrxR in mammals are expressed as dedicated isoforms for either predominantly cytosolic (Trx1 and TrxR1) (Fig. 6) or mitochondrial (Trx2 and TrxR2) localization.[65] Thioredoxin plays multiple functions in the cell that include the providing of reducing equivalents for DNA synthesis through ribonucleotide reductase (RR) enzyme and for reactive oxygen species (ROS) scavenging through the peroxiredoxins (Prxs). In addition, thioredoxin is found to reduce, and thus activate, a number of transcription factors that are all involved in the regulation of various aspects of cell growth and cell survival, including NF-κB,[66] AP-1,[67] SP-1, and p53.[68]

Expression levels of the cytosolic thioredoxin isoform Trx1 are increased in several human carcinomas[69,70] and linked to tumor aggressiveness, and to inhibition of apoptosis.[71,72] Hence, there is great interest in the thioredoxin system as a potential target for new anticancer drugs. Both gold(I) and gold(III) compounds are found to interact strongly with the thioredoxin system as detailed below.

As auranofin reacts mainly with thiol and/or selenol groups, it behaves as a potent and specific inhibitor of both cytosolic[73] and mitochondrial TrxR,[62,63,74] even in the nanomolar range. In addition to inhibition of mitochondrial TrxR, auranofin and other gold(I) compounds (e.g. aurothiomalate), at submicromolar concentrations, are shown to induce, in the presence of Ca^{2+} ions, mitochondrial swelling, mitochondrial membrane potential ($\Delta\psi m$) decrease, and stimulation of respiration, which is dependent on membrane permeability transition,[62] overall resulting in the release of cytochrome c into the cytoplasm.[63,75] This process is crucially involved in the formation of apoptosomes and the consequent induction of apoptosis.

The ability of gold(I) compounds, in particular auranofin, to inhibit TrxR and induce apoptosis has been recently investigated in cisplatin-resistant human ovarian cancer cells.[50] The treatment with auranofin of cisplatin-sensitive and -resistant ovarian cancer cells causes a consistent release of cytochrome c in both cell lines while cisplatin is effective only in the sensitive cells. Apoptosis is accompanied by the increased production of ROS. In resistant cells, hydrogen peroxide production is counteracted by a large overexpression of TrxR. Thus, these authors suggest that auranofin, acting as a potent inhibitor of TrxR, determines an alteration of the redox state of the cell, leading to increased production of hydrogen peroxide and to oxidation of the components of the thioredoxin system, which creates the conditions for augmented apoptosis.[50] Similar studies were recently carried out by Susan Berners Price in a group of related gold(I) compounds; the main results of those studies and their unified interpretation are summarized in a review.[7] The bis-chelated Au(I) bidentate phosphine complex of the water soluble ligand 1,3-*bis*(di-2-pyridylphosphino)propane (d2pypp), $[Au(d2\text{-}pypp)_2]Cl$,[5] as well as a number of gold(I) *N*-heterocyclic carbene complexes of the type $[(R_2Im)_2Au]^+$[76] are shown to selectively induce apoptosis in MDA-MB-468 human breast adenocarcinoma cells rather than in human normal breast cells (HMEC). Apoptosis is induced via the mitochondrial pathway by inhibition of both Trx and TrxR.[5] The selective induction of apoptosis in cancer cells represents an element of particular value.

Similarly to gold(I) compounds, gold(III) compounds are known to target, rather strongly and selectively, thiol and imidazole groups of proteins (as well as selenol groups).[9,27] Some gold(III) compounds developed by Cinellu et al.[77–80] are shown to inhibit mitochondrial TrxR2 and to greatly perturb mitochondrial functions.[63] The presumed site of interaction is the selenol moiety present in the active site of the carboxy terminus of the enzyme[63] as well as other cysteine and histidine residues. These compounds, in particular Aubipy and Aubipyxil, trigger mitochondrial swelling, although to a lesser extent than auranofin, probably in relation to their different permeability characteristics.[63] Also, $\Delta\psi m$ is scarcely diminished by this kind of compounds.

Later on, a series of gold(III) compounds characterized by an increasing number ($n = 0$–3) of carbon–gold bonds have been reported to inhibit TrxR.[69] Overall, the IC_{50} values for TrxR activity and for the TrxR/Trx system of the nine compounds studied ranged from 0.0022 to 1.8 μmol/L and from 0.18 to >50 μmol/L, respectively. The compound with two carbon–gold bonds is the most potent inhibitor of TrxR ($IC_{50} = 0.0022$ μmol/L).

$[Au[DMDT)X_2]$ and $[Au(ESDT)X_2]$ have been shown to inhibit both cytosolic and mitochondrial TrxR and, interestingly, the first (IC_{50} values of four different compounds ranging from 5.67 to 17.01 nM) more effectively than the second (IC_{50} values of four different compounds ranging from 24.74 to 35.87 nM).[81]

The high selectivity of gold compounds for selenoenzymes is also suggested by the fact that the activity of glutathione reductase, a protein structurally and functionally related to TrxR but lacking selenol at the catalytic site and relying upon sulfhydryls for its catalysis, requires greater concentrations of gold compounds to be inhibited.[73] In conclusion, TrxR seems to be a very specific target of gold(III) compounds as, in the same range of

concentrations, these compounds are almost completely ineffective in altering the respiratory chain.[63]

C. Activation of Downstream Signalling Events Leading to Apoptosis

It has been shown that auranofin-mediated generation of ROS in human promyelocytic leukemia HL-60 cells is an early event in the activation of the apoptotic cascade. Increased ROS subsequently activate p38 MAPK, which transduces a signal to the initiator caspase to triggering further proapoptotic events, which lead to caspase-3 activation, poly-ADP-ribose polymerase 1 (PARP-1) degradation, DNA fragmentation, and cell death.[82]

Recently, a cationic gold(I) *N*-heterocyclic carbene complex, $[(iPr_2Im)_2Au]Cl$, characterized by intermediate lipophilicity, has been shown to accumulate selectively in the mitochondria of tumorigenic cells (PIL cells) driven by the $\Delta\psi m$. This gold(I) complex depolarizes the $\Delta\psi m$, depletes the ATP pool, and activates caspase-3 and caspase-9, leading to apoptosis.[83]

The induction of apoptosis by mitochondrial death pathways related to ROS has been demonstrated by Wang et al.[18] for gold(III) porphyrin 1a in HONE1 human nasopharyngeal carcinoma cells. These authors propose a specific and detailed model for this cellular mechanism: gold(III) porphyrin 1a directly causes depletion of the $\Delta\psi m$, leading to alteration of bcl-2 family proteins (in particular suppression of bcl-2), translocation of the apoptosis-inducing factor (AIF) nucleus, and release of cytochrome c. This last effect further activates caspase-9 and caspase-3, and subsequently produces PARP-1 cleavage. Oxidative stress is likely to affect the cytotoxicity of gold(III) porphyrin 1a by regulating $\Delta\psi m$.

In a successive study, the same authors identify differentially expressed proteins by comparing the protein alterations induced by either gold(III) porphyrin 1a or cisplatin treatment in the SUNE1 human nasopharyngeal carcinoma cell line.[84] A wide series of protein alterations were indeed detected in this cell line after both treatments. The main alterations were found in cellular structure and stress-related chaperone proteins and in those involved in ROS (e.g. stomatin-like 2, peroxiredoxin 1 and 6, thioredoxin), in enzyme proteins and translation factors (e.g. mitochondrial single-stranded DNA binding protein—mtSSB, splicing factor 17, peptidylprolyl isomerase F, cyclophilin F—CypF), in proteins that mediate cell proliferation or differentiation (e.g. cyclophilin A—CypA, porin isoform 1 voltage-dependent anion channel 1—VDAC1, calcyclin binding protein, Siah-interacting protein—CacyBP, Ras-related nuclear protein), and in proteins belonging to the internal degradation system (e.g. proteasome α type 3, proteasome β type 4, proteasome α type 6).

Gold(III) porphyrin 1a treatment causes cell cycle arrest initially at G2-M phase, then at G0-G1, and upregulation of the proapoptotic protein p53 in SUNE1 cells.[60] Further examination of the MAPK family members shows that transient activation of $p38^{MAPK}$ is involved in gold(III) porphyrin 1a-mediated cell death and that phosphorylation of $p38^{MAPK}$ induced by the gold compound is enhanced by increasing concentrations of H_2O_2.[85] Overall, a differential regulation of phosphotyrosine proteins showed up related to $p38^{MAPK}$ activation in gold(III) porphyrin 1a-induced signal transduction cascade.[85]

Based on the above reported observations, it is possible to suggest that treatment with gold(III) porphyrin 1a causes multiple effects, leading to apoptosis in the human nasopharyngeal carcinoma cell lines. The balance between pro- and antiapoptotic signals ultimately determines the survival or death of cancer cells.

The effects of gold(III) dithiocarbamate derivatives on the levels of antiapoptotic bcl-2 and proapoptotic bax in a panel of leukemia cell lines have also been analyzed.[86] Short treatment (18 hr) with $[Au(MSDT)Br_2]$ (dibromo[methyl *N*-(dithiocarboxy-kS,kS′)-*N*-methylglicinato] gold(III)) or

[Au(MSDT)Cl_2] (dichloro[methyl *N*-(dithiocarboxy-kS,kS′)-*N*-methylglicinato]gold(III)) is found to decrease bcl-2 and upregulate or induce bax in all cell lines examined.

Other gold(III) dithiocarbamate complexes, such as [Au[DMDT)X_2] and [Au(ESDT)X_2], have been shown to trigger ROS generation and ultimately increase the levels of phosphorylated ERK1/2 through the inhibition of both cytosolic and mitochondrial TrxR2, in human uterine cervical carcinoma HeLa cells.[81] The authors hypothesize that persistent ERK1/2 activation caused at first by the accumulation of hydrogen peroxide and afterward by the activation of ASK-1 may be responsible for cell death.

D. Inhibition of the Proteasome

The ubiquitin–proteasome pathway that is essential for many fundamental cellular processes including cell cycle regulation, apoptosis, angiogenesis, and differentiation has recently been investigated as a tumor target. Proteasome inhibitors are becoming the object of very intense research (Fig. 7).[87]

A gold(III) dithiocarbamate compound has been shown to inhibit, in a concentration-dependent manner, all the peptidase activities (chymotrypsin-like, trypsin-like, and PGPH-like) of a rabbit 20S purified proteasome (the proteolytic core of the 26S proteasome complex) with similar potencies ($IC_{50} = 7.4$, 10.2, and 7.0 μmol/L, respectively).[88] This compound also showed the same inhibitory effects in intact MDA-MB-231 breast cancer cells. Proteasome inhibition by this compound was confirmed by decreased proteasome activity, increased levels of ubiquitinated proteins, and the proteasome target protein p27. Most importantly, inhibition of the proteasome activity and accumulation of p27 were also found in MDA-MB-231 xenografts treated with this gold(III) dithiocarbamate compound. Induction of apoptosis by this gold(III) dithiocarbamate compound was confirmed either in the MDA-MB-231 cell line and in the treated tumors by multiple assays that measure characteristic cellular and biochemical hallmarks. Thus, the authors suggest that proteasome could be a primary target for gold(III) dithiocarbamates both in vitro and in vivo and that inhibition of the proteasomal activity by these compounds is associated with apoptotic cancer cell death.[88]

E. Inhibition of Protein Kinases

Protein kinase C (PKC), a family of structurally related protein kinases, is involved in a large variety of cellular functions, including cellular proliferation, cell cycle control, differentiation, polarity, and survival.[89] Altered PKC activity, localization, and/or expression have been observed in several tumor types.[90] To date, several PKC isozymes have been identified as potential therapeutic targets:[91,92] a number of isozyme-selective PKC inhibitors have been developed and some are already in clinical use. The atypical protein kinase C iota (PKCι) (Fig. 8) is a bona fide human oncogene that is required for the transformed growth of human cancer cells.[93]

PKCι, as well as other atypical PKCs, is structurally and functionally distinct from other PKCs as its catalytic activity does not depend upon diacylglycerol, calcium, or phosphatidylserine[94,95] but may be regulated by 3-phosphoinositides,[96] phosphorylation by the phosphoinositide-dependent kinase,[97,98] and through specific protein–protein interactions.[93,99]

It has been observed that elevated expression levels of PKCι play an important role in the cell growth of nonsmall cell lung cancer (NSCLC) both in vitro and in vivo by the activation of a PKCι → Rac1 → Pak → Mek → Erk signalling axis.[100] The Phox Bem 1 (PB1) domain is a structurally conserved, protein–protein interaction domain that is present in a family of signalling molecules, including PKCι.

Upon considering the relevant role that cysteine residues usually play in interactions with gold compounds, it was investigated whether the PB1 domain interactions between the po-

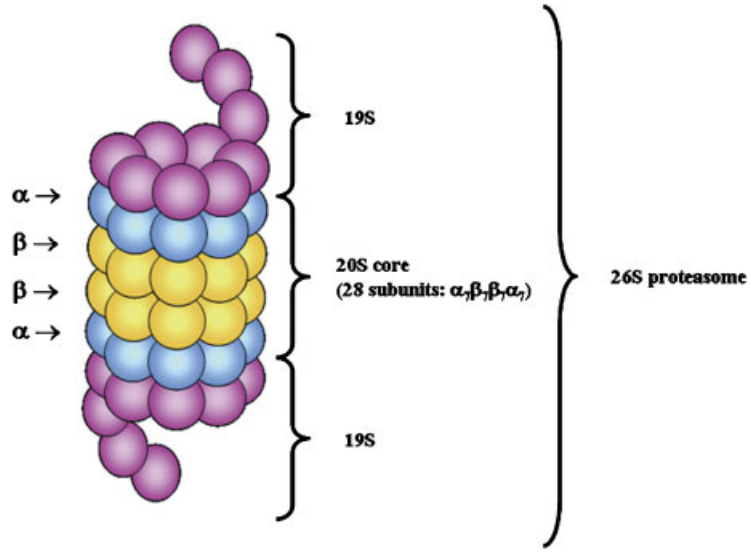

Figure 7. Schematic representation of the proteasome. It consists of a 28-subunit catalytic core (20S proteasome) and two multisubunit ATPase-containing PA700(19S) regulators. The 20S core is composed of two outer and two inner rings. Each of the two inner rings consists of seven different β-subunits, which contain the three different catalytic sites (caspase-like, trypsin-like, and chymotrypsin-like sites). Each of the two outer rings is composed of seven different α-subunits, none of which have catalytic activity, but which serve as an anchor for the 19S regulators.

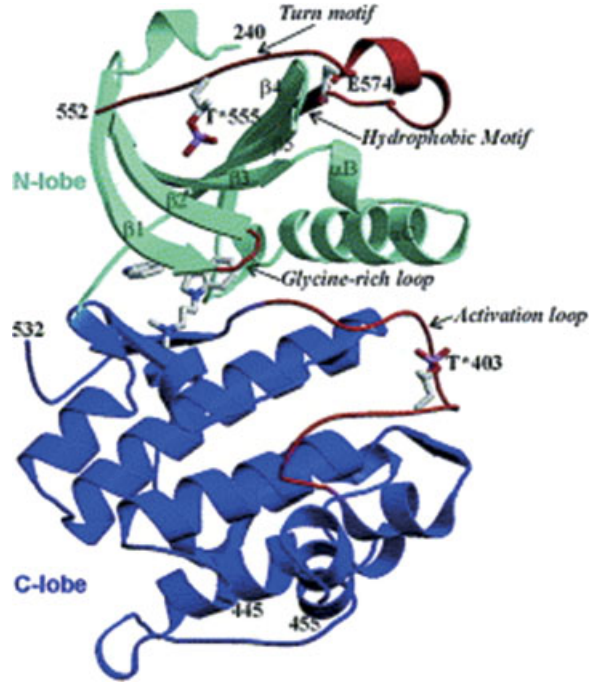

Figure 8. Ribbon plot of the PKCι catalytic domain structure. The N-lobe is cyan and the C-lobe is blue. The glycine-rich loop, activation loop, turn motif, and HM segment are displayed in red. BIM1 and phosphorylated residues are given in stick representation (reprinted from Messerschmidt et al., J Mol Biol 2005;352:918–931. Copyright 2005, with permission from Elsevier). PKCι, protein kinase C iota.

larity protein par6 containing cysteine residue and PKCι, in human A549 NSCLC cells, might represent a potential target for aurothiomalate and aurothioglucose.[93,101,102] Results indicate that both compounds act as potent inhibitors of PKCι-par6 interaction in vitro ($IC_{50}\sim 1\,\mu M/L$). Aurothioglucose blocks PKCι-dependent signalling to Rac1 and inhibits transformed growth of NSCLC cells.[102] Aurothiomalate can form gold–cysteine adducts on target cellular proteins, in particular with a critical cysteine residue, Cys69, located within the active site of the enzyme. The adduct at Cys69 protrudes into the binding cleft normally occupied by par6. These authors conclude that the selective targeting of Cys69 within the PB1 domain of PKCι might explain the inhibition of cellular transformation by aurothiomalate.[93,101]

5. PRECLINICAL STUDIES

A. In Vitro Studies

Early studies indicate that auranofin has in vitro anticancer activity similar to that of cisplatin.[103,104] A systematic investigation of the cytotoxic activity of auranofin and a variety of its analogues in murine P388 leukemia and B16 melanoma cell lines shows that compounds containing both phosphine and thioglucose ligands are the most potent ($IC_{50} \leq 10\,\mu M$).[104]

Another phosphine gold compound, μ-[1,2-*bis*(diphenylphosphino) ethane] *bis*[(1-thio-β-D-glucopyranosato-S)gold(I)] complex with IC_{50} values in the micromolar range (from 4 to 11 μM) toward a panel of tumor cisplatin-sensitive cell lines,[105] displays similar cytotoxic activity even in cisplatin-resistant cell lines.[106]

Gold phosphine compounds containing a variable number of coordinated phosphorus atoms (from 1 to 4) show increased cytotoxic potency in murine tumor cell lines as the number of gold-coordinated phosphorus atoms increases.[107,108]

Unfortunately, after relatively encouraging studies on selected tumor cell lines,[109–112] auranofin analogues containing thiolate ligands derived from thionucleobases (phosphine gold(I) thionucleobase compounds) have not demonstrated marked cytotoxic activity when screened through the National Cancer Institute's panel of 60 tumor cell lines.[113] However, related phosphine gold(I) thiolates show higher cytotoxic activity compared with cisplatin and 5-fluorouracil in seven human solid tumor cell lines.[114]

Gold(I) dithiocarbamate derivatives (DMDTM gold derivatives) have no or modest cytotoxic activity against highly sensitive human promyelocytic leukemia HL-60 cells.[16]

At present, the cytotoxic activity of a relatively wide number of gold(III) complexes has been evaluated. The possibility of combining gold(III) compounds with nitrogen-containing ligands derived from biologically active molecules such as streptonigrin,[115] uracil,[116] and glycylhistidine[56] has led to the synthesis of several gold(III) compounds. The gold(III) streptonigrin complex shows inhibitory values of 0.05 mg/mL against P388 leukemia cells.[115] Gold(III) compounds containing uracil derivatives show growth inhibition values ranging from 11.0 to 74.5% in the HeLa cell line.[116] The chloroglycylhistidinate gold(III) compound has been investigated both in cisplatin-sensitive and -resistant human ovarian carcinoma cell lines (A2780/S and A2780/R, respectively) and is more active than cisplatin in the resistant cell line.[56]

A wide series of compounds containing imine and amine ligands have also been assayed. Gold compounds synthesized by Cossu et al.[117] show higher cytotoxic activity than carboplatin both in A2780/S and A2780/R and mouse leukemia (L1210/S and L1210/R) cell lines. Cisplatin is more active than gold compounds in both sensitive cell lines (Table I).

Square planar gold(III) complexes containing at least two gold–chloride bonds in *cis*-position produce significant cytotoxic effects in the human cisplatin-resistant CCRF-CEM/R (leukemia) and A2780/R lines.[118] Some compounds are also more active than cisplatin in the A2780 cisplatin-sensitive cells (Table II).

The A2780/S and A2780/R cell lines have also been used to evaluate the cytotoxic effects of a series of classical gold(III) complexes (Table III).[15] Most of these compounds manifest a relevant cytotoxicity with IC_{50} values generally falling in the low micromolar range. In particular, $[Au(terpy)Cl]Cl_2$ turns out to be more active than cisplatin both in A2780/S and in A2780/R and $[Au(phen)Cl_2]Cl$ only in the resistant cell line (Table III).

Cytotoxic effects of several organogold(III) compounds have also been analyzed. Organogold(III) DAMP were the first ones studied.[119,120] At a primary screening conducted against a panel of seven human tumor cell lines, $[AuCl_2(damp)]$ produced cytotoxic effects similar to those of cisplatin with the breast carcinoma ZR-75-1 cell line being the most sensitive (IC_{50} 11 μM).[119] However, four additional organogold(III) DAMP compounds failed to show higher activity than cisplatin both in cisplatin-sensitive and -resistant cell lines (Table IV).[120]

Bipyridyl gold(III) compounds have relevant cytotoxic effects against A2780/S, A2780/R, SKOV3 (this latter inherently resistant to cisplatin) human ovarian carcinoma cell lines and the CCRF-CEM cell line, either sensitive (CCRF-CEM/S) or resistant (CCRF-CEM/R) to cisplatin. In particular, $[Au(bipy^{c}\text{-}H)(OH)][PF_6]$ is markedly more active than cisplatin in A2780/R (Table V).[53]

Table I. In Vitro Growth Inhibition (IC_{50} μM) of Tumor Cell Lines by Gold(III) Compounds Containing Imine Ligands (General Formula $[Au(NR^{n})Cl_3]$[117]

Compound	A2780/S	A2780/R	L1210/S	L1210/R
$[Au(NR^{10})Cl_3]$	14.3	31.2 (2.2)	19.4	18.3 (0.94)
$[Au(NR^{11})Cl_3]$	22.0	23.4 (1.1)	12.8	11.2 (0.87)
$[Au(NR^{12})Cl_3]$	14.9	16.9 (1.1)	14.0	12.4 (0.88)
Cisplatin	8.3	55.7 (6.7)	3.3	28.7 (8.7)
Carboplatin	119.9	894.8 (7.5)	67.6	258.8 (3.8)

( ) resistance index = IC_{50}(resistant line)/IC_{50}(sensitive line). A2780, human ovarian carcinoma; L1210, murine leukemia; S, sensitive to cisplatin; R, resistant to cisplatin.

Table II. In Vitro Growth Inhibition (IC_{50} μM) of Tumor Cell Lines by Gold(III) Compounds Containing At Least Two Gold–Chloride Bonds[a] [118]

Compound	A2780/S	A2780/R	SKOV3	HEC1-A	HCT-8	CCRF-CEM/S	CCRF-CEM/R
$AuCl_3$(Hpm)	10.10	21.0 (2.1)	30.50	21.50	29.0	17.80	36.30 (2.0)
$AuCl_2$(pm)	6.90	16.30 (2.4)	33.30	17.30	28.50	23.30	36.30 (1.5)
$AuCl_2$(mesal)	2.80	3.70 (1.3)	13.20	11.80	11.60	2.0	13.60 (6.8)
$AuCl_2$(esal)	2.10	3.80 (1.0)	13.50	14.10	8.0	5.0	10.80 (2.2)
$Na[AuCl_4]$	11.0	17.70 (1.8)	42.0	12.60	26.50	22.50	35.50 (1.6)
Cisplatin	5.30	41.70 (7.9)	24.50	6.40	3.90	1.50	44.80 (29.9)

( ) resistance index = IC_{50}(resistant line)/IC_{50}(sensitive line). A2780, SKOV3, human ovarian carcinoma; HEC1-A, human endometrial carcinoma; HCT-8, human colon cancer; CCRF-CEM, human leukemia; S, sensitive to cisplatin; R, resistant to cisplatin.

[a]72-hr drug exposure.

Table III. In Vitro Growth Inhibition (IC_{50} μM) of Tumor Cell Lines by Gold(III) Compounds Containing Amine Ligands[a] [15]

Compound	A2780/S	A2780/R
$[Au(en)_2]Cl_3$	8.36	17.0 (2.03)
$[Au(dien)Cl]Cl_3$	8.2	18.7 (2.28)
$[Au(cyclam)ClO_4]Cl$	99.0	>120
$[Au(terpy)Cl]Cl_2$	0.2	0.37 (1.23)
$[Au(phen)Cl_2]Cl$	3.8	3.49 (0.92)
Cisplatin	1.2	14 (11.6)

( ) resistance index = IC_{50}(resistant line)/IC_{50}(sensitive line). A2780, human ovarian carcinoma; S, sensitive to cisplatin; R, resistant to cisplatin.

[a]72-hr drug exposure.

Table IV. In Vitro Growth Inhibition (IC_{50} μM) of Tumor Cell Lines by Organogold(III) Compounds With the General Formula $[AuX_2(damp)]$ [120]

Cell line	X = Cl	X = SCN	X = CH_3CO	X = $O_2CCH_2CCO_2$	X = $O_2C_2O_2$	Cisplatin
Primary panel[a]						
SW620	124.0	51.0	281.0	205.0	67.0	167.0
SW1116	119.0	47.0	238.0	215.0	80.0	163.0
ZR-75-1	27.0	34.0	45.0	41.0	36.0	27.0
HT29/219	55.0	25.0	67.0	19.0	36.0	17.0
HT1376	30.0	6.7	13.0	10.0	11.0	23.0
SKOV3	45.0	20.0	13.0	10.0	11.0	23.0
Ovarian carcinoma cell line panel[b]						
HX62	57.0	31.0	34.0	30.0	27.0	18.0
SKOV3	109.0	39.0	107.0	42.0	30.0	5.2
CH1/S	13.0	10.0	11.0	11.0	2.7	0.12
CH1/R	22.0 (1.7)	11.0 (1.1)	12.0 (1.1)	13.0 (1.2)	3.3 (1.2)	0.56 (4.7)
A2780/S	8.2	2.0	3.5	3.7	2.7	1.2
A2780/R	47.0 (5.7)	26.0 (13)	35.0 (10)	35.0 (9.5)	16.0 (5.9)	10.0 (8.3)

( ) resistance index = IC_{50}(resistant line)/IC_{50}(sensitive line). SW620, SW1116, SW403, HT29/219, human colon carcinoma; ZR-75-1, human breast carcinoma; HT1376, human bladder carcinoma; A2780, SKOV3, HX62, CH1, human ovarian carcinoma; S, sensitive to cisplatin; R, resistant to cisplatin.

[a]4-hr drug exposure.

[b]96-hr drug exposure.

Four gold(III) compounds (AuXyl, AuTol, AuPyAcO, and AuPzCl) have been screened in a panel of five human tumor cell lines. After 72-hr drug exposure, AuPyAcO and AuXyl were generally the most active, showing the highest cytotoxic activity against A2780/S (Table V).[19] After shorter drug exposure (48 hr), the same compounds and $[Au(bipy^{dmd}\text{-}H)(OH)][PF_6]$ showed IC_{50} values comparable to those of cisplatin both in A2780/S and in A2780/R cell lines.[20]

Another series of dinuclear gold(III) compounds with bipyridyl ligands (Auoxo1–6) have also been evaluated in A2780/S and A2780/R.[55] Auoxo6 is more active than cisplatin against both cell lines (Table VI).[55]

Differently from gold(I) dithiocarbamate compounds, gold(III) dithiocarbamates display a very promising cytotoxic profile.[16] Four DMDT and ESDT gold(III) derivatives

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Table V. In Vitro Growth Inhibition (IC_{50} μM) of Tumor Cell Lines by Mononuclear Organogold(III) Compounds After a 72-hr Drug Exposure[19,53]

Compound	A2780/S	A2780/R	MCF7	HT29	A549	SKOV3	CCRF-CEM/S	CCRF-CEM/R
$[Au(bipy)(OH)_2][PF_6]$	8.80	24.10 (2.7)	–	–	–	34.40	52.90	58.60 (1.1)
$[Au(bipy^{c}\text{-}H)(OH)][PF_6]$	3.30	8.20 (2.5)	–	–	–	13.30	11.90	51.20 (4.3)
$[Au(bipy^{dmb}\text{-}H)(OH)][PF_6]$	8.20	12.70 (1.6)	35.30	24.60	>50	–	–	–
AuPzCl	30.20	31.30 (1.0)	33.70	>50	>50	–	–	–
AuPyAcO	2.90	6.40 (2.2)	17.70	8.60	~49	–	–	–
AuXyl	2.50	5.70 (2.3)	5.20	~25	~35	–	–	–
AuTol	6.15	14.30 (2.3)	18.10	~25	~35	–	–	–
Cisplatin	1.30	15.30 (11.7)	5.30	6.30	–	21.60	1.0	14.10

( ) resistance index = IC_{50}(resistant line)/IC_{50}(sensitive line). A2780, SKOV3, human ovarian carcinoma; MCF7, human breast cancer; HT29, human colon cancer; A549, human nonsmall cell lung cancer; CCRF-CEM, human leukemia; S, sensitive to cisplatin; R, resistant to cisplatin.

exhibit relevant cytotoxic activities toward a panel of human tumor cell lines, being far more potent than cisplatin even at nanomolar concentration (Table VII). In addition, all the tested gold(III) complexes are more cytotoxic than cisplatin on cisplatin-resistant cell lines, with activity levels comparable to those induced on the parental sensitive cell lines, ruling out the occurrence of cross-resistance phenomena (Table VII).[16]

The same group of authors have recently investigated the cytotoxic properties of two gold(III) methylsarcosinedithiocarbamate derivatives on a panel of human acute myeloid leukemia (AML) cells.[86] [Au(MSDT)Cl_2] and [Au(MSDT)Br_2] are significantly more active than cisplatin in inhibiting the clonogenic growth of all AML cell lines in a dose-dependent manner.

Table VI. In Vitro Growth Inhibition (IC_{50} μM) of Tumor Cell Lines by Dinuclear Organogold(III) Compounds After a 72-hr Drug Exposure[55]

Compound	A2780/S	A2780/R
Auoxo1	22.80	23.30 (1.0)
Auoxo2	12.10	13.5 (1.1)
Auoxo3	25.40	29.8 (1.2)
Auoxo4	12.70	19.8 (7.3)
Auoxo5	11.0	13.2 (1.2)
Auoxo6	1.79	4.81 (2.7)
Cisplatin	2.1	24.4 (11.6)

( ) resistance index = IC_{50}(resistant line)/IC_{50}(sensitive line). A2780, human ovarian carcinoma; S, sensitive to cisplatin; R, resistant to cisplatin.

Table VII. In Vitro Growth Inhibition (IC_{50} μM) of Tumor Cell Lines by Gold(III) Compounds Containing Dithiocarbamate Ligands After a 24-hr Drug Exposure[16]

	[(DMDT)$AuCl_2$]	[(DMDT)$AuBr_2$]	[(ESDT)$AuCl_2$]	[(ESDT)$AuBr_2$]	Cisplatin
HELA	2.10	3.50	8.20	7.60	15.60
HL-60	0.80×10^{-2}	0.10×10^{-2}	2.0	0.14	0.35
Daudi	0.10×10^{-2}	0.10×10^{-2}	4.65	5.80	95.0
MeWo	2.0	0.10×10^{-2}	12.50	10.0	48.0
LoVo	2.40×10^{-2}	3.80	7.60	7.90	56.0
A549	0.35×10^{-2}	0.41	4.73	9.60	35.0
2008	0.20×10^{-2}	30.10	49.30	16.50	43.20
C13*	0.10×10^{-2}	2.18	23.80	0.10×10^{-2}	556.0
A431	1.20×10^{-2}	1.80	0.29	1.5×10^{-2}	77.40
A431-R	0.20×10^{-3}	2.80	0.43	0.10×10^{-2}	382.0
U20S	4.80	18.0	5.80	0.49	35.0
U20S-R	6.40	13.0	5.20	0.24	85.0

HELA, human squamous cervical adenocarcinoma; HL-60, human leukemic promyelocytes; Daudi, human Burkitt's lymphoma; MeWo, human malignant melanoma; LoVo, human colon adenocarcinoma; A549, human nonsmall cell lung adenocarcinoma; 2008, cisplatin-sensitive human ovarian carcinoma; C13*, cisplatin-resistant human ovarian carcinoma; U20S, cisplatin-sensitive human osteosarcoma; U20S-R, cisplatin-resistant human osteosarcoma; A431, cisplatin-sensitive human squamous cervix carcinoma; A431-R, cisplatin-resistant human squamous cervix carcinoma.

Gold(III) mesotetraarylporphyrin complexes (including gold(III) porphyrin 1a)[17] have been investigated in a panel of human tumor cell lines and have IC_{50} values ranging from 0.1 to 1.5 μM. Lack of cross-resistance with cisplatin was also observed. More recently, the in vitro cellular pharmacology properties of gold(III) porphyrin 1a have been more extensively investigated in a nasopharyngeal carcinoma cell line (SUNE1),[60] where the higher activity of the gold compound compared with cisplatin was confirmed.

B. Efficacy Data in In Vivo Studies

Although most gold(I) compounds exhibit marked antitumor activity in vitro, very limited success has been observed in vivo.[121] Efficacy data in mice inoculated with lymphocytic leukemia P388 are available for auranofin. Eight dose schedules ranging from 6 mg/kg every fourth day to 6 mg/kg twice daily for 9 days administered i.p. were used.[122] Increased survival is correlated with drug dose amount and/or dose frequency. The median survival times for the groups of animals treated at higher and more frequent doses are 18.5 days (3 mg/kg, twice daily), 21 days (4.5 mg/kg, twice daily), and 22 days (6 mg/kg, twice daily) with T/C (tumor/control) ratios of 185, 210, and 220%, respectively. The efficacy of auranofin in the same tumor model was only partially confirmed in the study of Mirabelli et al.[121] where the optimal dose of 12 mg/kg administered i.p. (days 1–5) produced a 59% increased life span (ILS); the drug was completely inactive when administered i.v., s.c., or p.o. Although several mouse models, including solid cancers (e.g. lung, colon, breast cancers), were used in this study, only the P388 leukemia model was sensitive to auranofin.

Other gold(I) thiolates, such as aurothioglucose and aurothiomalate, have been examined for antitumor activity. They inhibited the growth of the primary tumor and reduced lung metastases in mice bearing Lewis carcinoma.[123] Aurothiomalate was less active compared with cisplatin in Balb/C mice inoculated (i.p.) with syngenic Meth/A cells, although it displayed a wider range of dose effectiveness and no significant toxicity when administered in doses up to 125 mg/kg/day.[124]

Aurothioglucose has been evaluated in athymic nude mice injected with A549 NSCLC cells. Once tumors were established, mice were randomized to receive aurothioglucose 20 mg/kg or diluent (0.9% saline). In the presence of aurothioglucose, A549 cell tumors exhibited a marked reduction in growth kinetics compared with controls. However, results indicate that aurothioglucose has a cytostatic rather then cytotoxic effect on A549 tumors.[102]

The in vivo data available for gold(III) compounds as anticancer agents are even more limited, probably due to the high redox potential and relatively poor kinetic stability of several of these compounds under physiological conditions. However, a conspicuous number of gold(III) compounds that are stable against demetallation and/or reduction under physiological conditions are today available (see "Chemistry of gold compounds" section) and their in vivo evaluation is warranted.

Efficacy and toxicity data are now available for the gold(III) porphyrins developed by Che and co-workers[125] and for the gold(III) dithiocarbamates developed by Fregona and coworkers.[88] The efficacy of gold(III) porphyrin 1a has been evaluated in an orthotopic rat hepatocellular carcinoma model.[125] After an intratumoral injection of 0.5 or 0.75 mg/kg of gold(III) porphyrin 1a, followed by i.p. injections twice weekly to animals according to body weight until their death, survival of both groups of treated animals was significantly prolonged compared with that of the control group (median 42 and 40 vs. 30 days, $P<0.05$).[125] Interestingly, the tumor tissue of the treated rats contained larger areas of necrosis compared with those observed in the control group and the gold compound did not cause observable necrosis in the adjacent normal liver tissue. This last observation was

confirmed by plasma AST levels that were lower in the treated animals compared with the control group.[125]

In a recent study[88] the s.c. treatment of MDA-MB-231 tumor-bearing nude mice with a gold(III) dithiocarbamate compound significantly inhibited tumor growth, as a consequence of proteasomal inhibition and apoptosis induction. During the 29-day treatment with this compound at 1–2 mg/kg/day, no toxicity was observed, and mice did not display signs of weight loss, decreased activity, or anorexia. However, these toxicity data are preliminary and warrant further investigation.

Other in vivo experiences have been reported for 2-[(dimethylamino) methyl]phenyl-gold(III) compounds whose general formula is $[(CR^1)AuX_2]$. One of these compounds in which X corresponds to SCN was evaluated against murine ADJ/PC6 plasmacytoma; only 24% of tumor growth was inhibited at a dose of 25 mg/kg (the maximum tolerated dose). Two other compounds in which X represents the O_2CMe, acetate group and X_2 the $O_2CCH_2CO_2$, malonate group show activity comparable to that displayed by cisplatin in a bladder tumor (HT1376) xenograft. The compound characterized by the acetate group shows some antitumor activity also in an ovarian tumor (CH1) model.[120]

C. Animal Pharmacokinetic Data

There is little pharmacokinetic data on gold(I) compounds in animals and none currently available for gold(III) complexes.

The pharmacokinetics of MU-Gold, tetrakis (trishydroxymethyl) phosphine gold(I) chloride, active in a series of in vitro and in vivo tumor models, have been studied in normal dogs in anticipation of trials in cancer-bearing dogs.[126] MU-Gold (10 mg/kg) was administered by i.v. injection to three purpose-bred dogs. A two-compartment i.v. bolus model with first-order kinetics, mean elimination half-life of approximately 40 hr, and mean volume of distribution of 0.6 L/kg was established. Serum gold concentrations ranging from 10 to 50 μg/mL were sustained from 2 to 3 days with no clinically significant toxicities observed.

Gold sodium thiomalate was administered to New Zealand white rabbits by two different routes. A single 2 mg/kg dose of the drug was administered i.m. and i.v. to four and three animals, respectively.[127] The blood concentration–time profiles were described by a two-compartment open model with first-order absorption by intramuscular route. Gold was absorbed rapidly with a mean absorption half-life of 9.0 min and a peak concentration of 6.0 ± 1.0 μg/mL ($n = 4$). Blood concentrations declined in a biphasic manner; the mean α half-lives were 0.738 and 1.78 hr for the i.v. and i.m. routes, respectively. The corresponding terminal (β) half-lives were 54.1 and 63.0 hr. The estimated volume of the central compartment (70–93 mL/kg) agreed closely with the rabbit blood volume. The mean ($\pm$SD) extent of the dose absorbed following i.m. injection was $68.9 \pm 12.4\%$.

6. CLINICAL INVESTIGATIONS

To date, gold compounds have not been formally investigated as anticancer drugs in the clinic and no published clinical data are available (PubMed, American Society of Clinical Oncology—ASCO Proceedings, American Association of Cancer Research—AACR Proceedings, reviewed in December 2008). The Mayo Clinic web site (http://clinicaltrials.mayo.edu) reports an ongoing phase I study to evaluate the side effects and the best dose of gold sodium thiomalate administered to patients with NSCLC as well as the effect of this compound on PKCι expression.

7. CONCLUDING REMARKS

Gold compounds are undoubtedly an interesting class of metal compounds, which have great potential as cytotoxic and anticancer agents. A variety of structurally different gold compounds have been prepared and characterized during the last two decades with unique chemistry at the gold center and pronounced reactivity with a variety of biomolecules. There are evident differences between gold(I) and gold(III) compounds, both in terms of chemistry and biological activity. Their biological properties appear to be critically governed by the coordination environment of the metal center. Extensive data are available on the in vitro cytotoxic activity of several gold compounds and their pronounced growth inhibition in a variety of cell lines. Biochemical and cellular studies indicate a large variety of molecular mechanisms for the numerous cytotoxic gold compounds that are presently under investigation. Some major molecular and cellular targets have been identified. Nevertheless, only a few in vivo studies are available on these compounds at present. It is evident that more extensive in vivo investigations are warranted for gold compounds to assess their possible efficacy as anticancer agents more thoroughly.

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