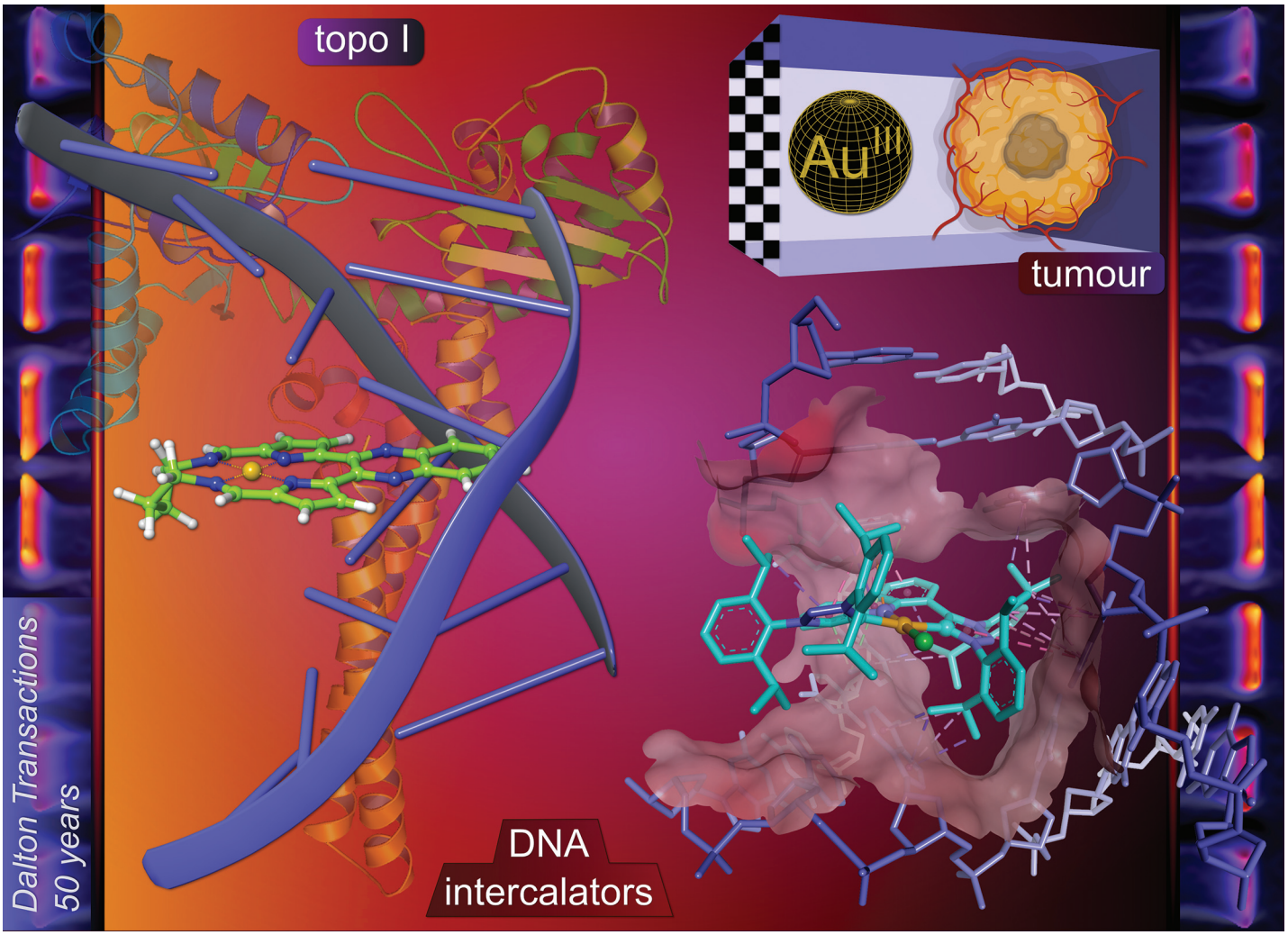

Showcasing research from Professor Orde Munro and Daniela Bezuidenhout's laboratories at the Molecular Sciences Institute, University of the Witwatersrand, Johannesburg, South Africa, and Laboratory of Inorganic Chemistry, Environmental and Chemical Engineering, University of Oulu, Oulu, Finland.

Cancer molecular biology and strategies for the design of cytotoxic gold(I) and gold(III) complexes: a tutorial review

This review addresses cancer molecular biology, historical gold metallodrug development, DNA-targeting drugs, and strategies to activate Au(I) and Au(III) with ligands for cell uptake, protein and nucleic acid binding, and imaging.

The illustration highlights two different designs (and thus different biomolecular targets) of cytotoxic Au(III) DNA intercalators from the Munro and Bezuidenhout laboratories.

As featured in:

See Daniela I. Bezuidenhout, Orde Q. Munro *et al.*, *Dalton Trans.*, 2021, **50**, 17413.

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Cancer molecular biology and strategies for the design of cytotoxic gold(I) and gold(III) complexes: a tutorial review

Danielle van der Westhuizen, [a] Daniela I. Bezuidenhout *[b] and Orde Q. Munro *[a]

This tutorial review highlights key principles underpinning the design of selected metallodrugs to target specific biological macromolecules (DNA and proteins). The review commences with a descriptive overview of the eukaryotic cell cycle and the molecular biology of cancer, particularly apoptosis, which is provided as a necessary foundation for the discovery, design, and targeting of metal-based anticancer agents. Drugs which target DNA have been highlighted and clinically approved metallodrugs discussed. A brief history of the development of mainly gold-based metallodrugs is presented prior to addressing ligand systems for stabilizing and adding functionality to bio-active gold(I) and gold(III) complexes, particularly in the burgeoning field of anticancer metallodrugs. Concepts such as multi-modal and selective cytotoxic agents are covered where necessary for selected compounds. The emerging role of carbenes as the ligand system of choice to achieve these goals for gold-based metallodrug candidates is highlighted prior to closing the review with comments on some future directions that this research field might follow. The latter section ultimately emphasizes the importance of understanding the fate of metal complexes in cells to garner key mechanistic insights.

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Background

Since cancer is predicted to become the leading cause of mortality in every region of the world,[1,2] the quest for effective, yet selective anticancer therapy remains paramount. In this tutorial review, we aim to provide a brief description of the general molecular mechanism of cancer and its fundamental origin in genomic instability. Cancer cells are not only associated with

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an increased rate of proliferation but are further endorsed by their ability to evade cell death pathways.[3,4] Disrupting mitochondrial function, which often (but not exclusively[5,6]) initiates apoptosis,[7] has emerged as a key chemotherapeutic strategy to elicit cytotoxicity in cancer cells.[8,9] Currently, many anticancer drugs in clinical use (mitomycin C,[10] dactinomycin,[11] bleomycin,[12] chlorambucil,[13] doxorubicin,[14] *etc.*) target DNA[15,16] and/or the enzymes which regulate DNA function.[17] One such drug, cisplatin—a metallodrug,[18] has paved the way for the potential use of metal complexes as anticancer agents. The active design and development of metallodrugs reflects their structural diversity and electronic tunability.[19] To the drug developer, these advantages provide access to multiple macromolecular targets. This versatility is exemplified by the widespread exploration of Au(I) and Au(III) complexes as potential new chemotherapeutic agents.[20] Specifically, Au(I) complexes preferentially bind to cysteine (Cys) and selenocysteine (Sec) residues in proteins, with thioredoxin reductase (TrxR) being an especially suitable target for drug development as TrxR inhibition induces the mitochondrial apoptotic pathway.[21] Gold(III) complexes are often hampered by their instability under physiological conditions.[22–24] However, the use of highly basic C- and N-donor ligands to stabilize higher metal ion oxidation states,[25,26] allows metallodrug designers to routinely target crucial enzymes and DNA with Au(III) complexes. This review will follow the general rubric outlined in this paragraph, culminating in a critical analysis of the way forward for carbene complexes of Au(I) and Au(III).

Cancer biology: an introduction

Cancer is associated with irregularities in cell replication

The proliferation of healthy cells is controlled by their response to extracellular signals that influence the internal circuitry of the cell, *i.e.*, the cell cycle. For the duration of the cycle (Fig. 1), the cell grows (G_1 and G_2-phase), replicates DNA (S-phase) and divides into two genetically identical daughter cells (M-phase). Cells can withdraw from this cycle to enter a non-dividing state (G_0) as a response to intra- or extracellular signals to either temporarily halt proliferation, to differentiate and mature, or undergo cell death. The progression from one stage to the next within the cell cycle is tightly regulated by a cascade of protein kinases (cyclin-dependent kinases, CDKs) and a checkpoint control system.[27] The regulation of the cell cycle is crucial to ensure cell number homeostasis and maintenance of normal tissue function.

Protein kinases (such as CDKs) are enzymes that activate or inactivate other proteins and play significant roles in signal transduction pathways within cells. In the case of CDKs, these kinases themselves are only activated by proteins named cyclins (hence the term "cyclin-dependent kinase"). Cyclins are present at different concentrations at different phases of the cell cycle and therefore orchestrate the events of the cell cycle depending on the type, activity and concentration of specific CDK-cyclin complexes within the cell.[28,29] CDKs are themselves regulated by phosphorylation involving other kinases.[29]

The checkpoint control system evaluates if critical events such as replication of DNA or chromosome segregation occurred correctly and signals the cell cycle machinery to either stop or proceed with the cycle. Critical checkpoints are found in the G_1, G_2 and M phase, but the G_1/S checkpoint (Fig. 1) is considered to be the most important as cells that proceed past this checkpoint usually transit through the S, G_2 and M phase.[30] The cell responds to extracellular signals only in the G_1 phase, after which the progression to division is

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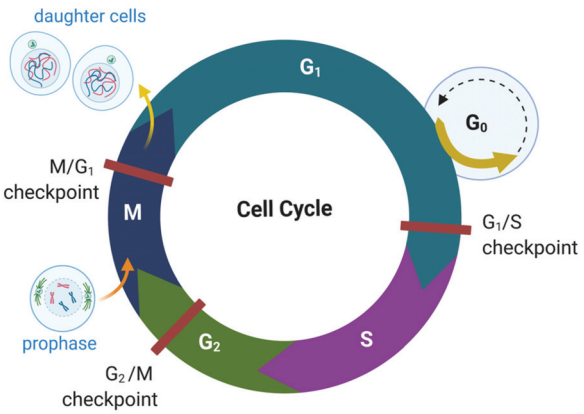


Fig. 1 Phases of the eukaryotic somatic cell cycle. The cell cycle describes the life of a cell from where it is initially formed from a dividing parent cell until the cell itself divides into two daughter cells. The cycle is divided into sequential phases namely G_1 (first gap), S (synthesis), G_2 (second gap), and M (mitotic). During the G_1 and G_2 phases, the cell grows and carries out functions such as forming new organelles and protein synthesis. Between these two phases, the cell undergoes DNA replication (S-phase) to prepare for cell division and cytokinesis (M-phase). If too few growth factors are available when the cell reaches the G_1/S checkpoint, the cell enters a quiescent stage, G_0. The illustration (and those presented in Fig. 2–4, 9 and Fig. 16) was created with BioRender.com.

autonomous. Cells that receive a stop signal at the G_1/S checkpoint withdraw from the cycle and transition to G_0.[30]

The lack of cell cycle regulation can result in limitless replicative potential and is therefore associated with the development of cancer.[28] Indeed, cancer cells can proceed through the G_1/S checkpoint independent of extracellular signals (both growth and antigrowth signals) and remain in the cycle to avoid terminal differentiation or cell death.[30] However, the progression from healthy cells to cancerous cells is a multistep process and is associated with many more acquired capabilities that are common to most types of cancer cells. Cancer cells can evade the immune system, generate neovascular support by the process of angiogenesis and can invade tissues and metastasize. Recent studies suggest that cancer cells can also reprogram cellular energy metabolism to sustain uncontrolled proliferation.[31,32]

The origin of cancer: genomic instability

Aside from the intrinsic genomic predisposition of certain cell types to become cancerous,[33,34] cancer cells typically achieve their characteristic hallmarks because of changes in their genomes. More specifically, the viability of a cancer cell relies on multiple mutations in the genes where the gene products are key players in maintaining the genomic stability in healthy cells.[31,32] Mutations in two types of genes have been identified in the pathogenesis of cancer, namely oncogenes and tumour-suppressor genes.

Proto-oncogenes, which are the nonmutated form of oncogenes, code for proteins that function in signal transduction pathways that stimulate cell growth and division. Mutations in proto-oncogenes (*e.g.*, the *ras* gene that codes for the Ras protein) usually leads to increased activity. The products of tumour suppressor genes, on the other hand, play key roles in signalling pathways that result in inhibited cell division. Mutations in tumour suppressor genes (*e.g.*, the TP53 gene that codes for the p53 protein) lead to a loss in function. Both scenarios can result in increased cellular proliferation and destabilize the genome.[27,28,31,32,35] For instance, in about 40% of human melanomas and 25% of human tumours, the MAPK (mitogen-activated protein kinase) signalling pathway is deregulated due to mutated proteins that play key roles in the pathway. The MAPK pathway is a signal transduction pathway that stimulates cellular proliferation and consists of a cascade of proteins, which are products of proto-oncogenes (Fig. 2). Ras proteins initiate the cascade by relaying signals that are received from an extracellular growth factor (*e.g.*, epidermal growth factor, EGF). In some cancers, mutated Ras can initiate the signalling cascade without the extracellular growth factor.

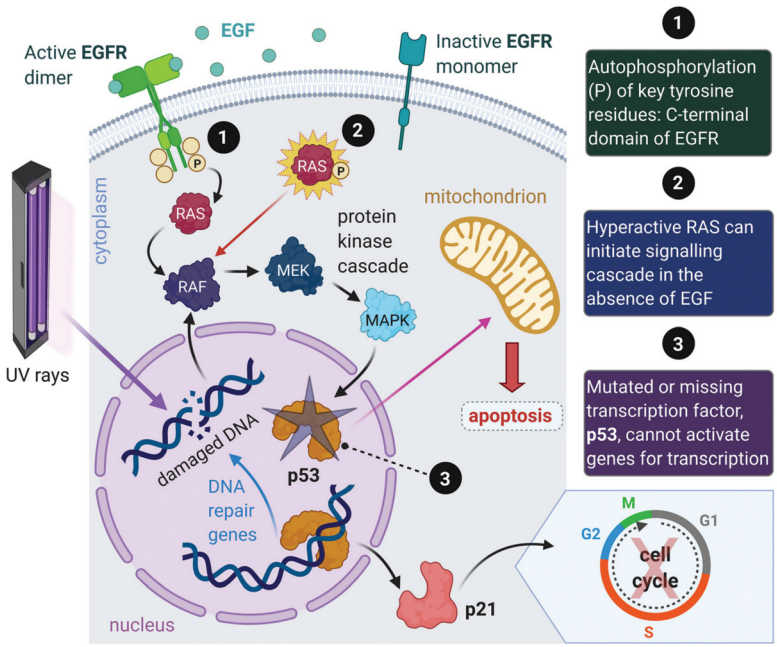


Fig. 2 A simplified representation of the MAPK cell signalling pathway. This pathway is triggered by the binding of external growth factor (*e.g.*, epidermal growth factor, EGF) to the EGF receptor (EGFR) on the membrane of the cell. Ras proteins (small GTPase signalling proteins) are activated by the receptor and initiate the signalling cascade involving a series of protein kinases. The last kinase (MAPK) activates a transcription factor (p53) that allows the synthesis of a protein (p21) that stimulates the cell cycle. (Note: *p21* transcription can be initiated independently of p53.) Excessive cell replication can occur because of a mutated Ras protein, as the pathway can be initiated without the binding of EGF. In the case of UV radiation induced DNA damage, p53 is activated and promotes either the transcription of genes directly involved in DNA repair, the transcription of p21 that can halt the cell cycle to prevent damaged DNA from being copied or induce apoptosis if the DNA is damaged beyond repair. If p53 is missing or defective due to a mutation in TP53, the response system fails to prevent the cell cycle from copying faulty genetic material and fails to induce apoptosis. Acronyms: RAF, proto-oncogene serine/threonine protein kinase; MEK; tyrosine/threonine protein kinase; MAPK, mitogen-activated protein kinase; P, inorganic phosphate.

Moreover, this activation can affect downstream transcription factors, which regulate a repertoire of cellular functions such as DNA replication, cell cycle progression, apoptosis, differentiation and proliferation.[36–38] Cancer cells can potentially acquire multiple hallmark capabilities such as sustained proliferative signalling, evasion of apoptosis, angiogenesis, modified metabolism and invasion by the mutations of these oncogenic genes.[32,36]

Furthermore, mutations in tumour suppressor genes (*e.g.*, TP53) and the subsequent inactivation of their products (*e.g.*, p53 protein) enable cancer cells to be insensitive to growth inhibitory signals, evade differentiation and avoid programmed cell death (apoptosis).[39] Tumour suppressors control cellular proliferation and inhibit tumour development. In more than half of human cancers, TP53 is mutated and leads to a loss of function in p53; this protein is an important transcription factor that prevents, as a response to damaged DNA, mutated DNA from being replicated by activating several genes (Fig. 2). The protein product (p21) of one such gene, *p21*, can halt the cell cycle by binding to CDKs to allow time for DNA repair to take place.[40] (Transcription of *p21* can also occur *via* a p53-independent pathway.[41]) Moreover, p53 activates DNA repair genes, however when the damage is irreparable, p53 initiates apoptosis by activating pro-apoptotic genes that induce cell death. Cells with damaged DNA or intracellular regulatory circuits have an increased frequency of mutations and loss of genomic stability.[27,31,32]

Triggering apoptosis: the key objective

The ability of cancer cells to develop into a tumour is not only attributed to a rise of uncontrolled proliferation, but also to the reduction in the rate of cell death. Although cancer cells can successfully evade cell death,[31] they are not fully immortal. The goal of cytotoxic anticancer drugs is to kill tumour cells directly, thereby decreasing the tumour mass,[42] while clinically, chemotherapy aims to reduce the tumour burden for the patient.

Apoptosis is a highly conserved mechanism of cell death that regulates the number of cells in the body. There are two general cell signalling pathways that induce apoptosis, namely the extrinsic and intrinsic pathway. The extrinsic pathway is initiated by extracellular death receptors and has a significant role in immune system functions. The intrinsic pathway, which is also known as the mitochondrial pathway, is of interest as this pathway is initiated intracellularly as a response to DNA damage, anticancer therapy, depleted growth factors and oxidative stress.[43,44]

These diverse apoptotic signals all converge on the mitochondria and stimulate changes in the mitochondria that result in mitochondrial outer membrane permeabilization (MOMP),[45] an event that induces apoptosis by either the release of pro-apoptotic proteins such as cytochrome *c* from the mitochondria or leads to the loss of vital mitochondrial functions (*e.g.*, respiration).[46] MOMP involves the opening of permeability transition (PT) pores located on the inner mitochondrial membrane (IMM) and is accompanied by a sudden increase in IMM permeability allowing ions and water to enter the matrix, leading to a loss in mitochondrial transmembrane potential and swelling of the matrix.[47,48] The outer mitochondrial membrane then ruptures[49,50] and releases pro-apoptotic proteins into the cytoplasm of the cell. The respiratory chain and production of ATP cease in response to MOMP as vital proteins leak out of the mitochondria, also leading to cell death.[51–53]

The release of the pro-apoptotic protein, cytochrome *c*,[54,55] initiates a signalling cascade of the major protein group involved in apoptosis namely caspases (Fig. 3). Cytochrome *c* binds to Apaf-1 (apoptosis protease-activating factor-1) facilitating the binding of procaspase-9, forming a star-shaped apoptosome (recently structurally elucidated by cryo-electron microscopy).[56] Procaspase-9 is cleaved to form active caspase-9, which subsequently activates procaspase-3, yielding the effector caspase (caspase-3). Effector caspases initiate apoptosis by activating proteins that are responsible for the cellular changes associated with apoptosis and are characterized by cell membrane blebbing, cell shrinkage, chromatin condensation and DNA fragmentation. Thereafter, the contents are packaged into apoptotic bodies and ingested by neighbouring or immune cells through phagocytosis.[35,51,53,57]

The Bcl-2 protein family are considered as the regulators of apoptosis but more specifically, they mediate and control the events leading to MOMP.[45] The Bcl-2 protein family consists of a group of proteins that all have at least one of the four relatively conserved Bcl-2 homology (BH) domains (Fig. 3 inset). Members of this protein family are either initiators (pro-apoptotic) or inhibitors (anti-apoptotic) of apoptosis. The pro-apoptotic members are further divided into BH3-only (*e.g.*, Noxa, Puma) and multi-domain members (*e.g.*, Bax, Bak), the latter sequestered or neutralized by anti-apoptotic members (*e.g.*, Bcl-2, Bcl-xL). The relative ratio of anti- and proapoptotic members work together to control cell death.[35,51–53]

Apoptosis is triggered once BH3-only proteins receive a death signal. For instance, in response to DNA damage,[58] p53 activates the BH3-only proteins, Noxa and Puma, to induce activation of downstream multi-domain proapoptotic proteins.[59,60] BH3-only proteins can release multi-domain proapoptotic proteins such as Bax and Bak from anti-apoptotic proteins (*e.g.*, Bcl-2). Unbound Bax and Bak subsequently permeabilize the OMM for the release of cytochrome *c* by either forming pores in the OMM themselves or opening the PT pores to induce MOMP (Fig. 3).[45,46] The anti-apoptotic proteins such as Bcl-2 maintain the mitochondrial membrane status to prevent the release of cytochrome *c*, balance the interactions between the members of the Bcl-2 family and counterbalance the damage done to the mitochondria, nucleus and endoplasmic reticulum (ER) by reactive oxygen species (ROS).[51,52]

Stress to the ER can also initiate apoptosis. The main function of the ER involves protein processing and sorting. The ER also plays a key role in the homeostasis of calcium,[61] as it is the major store of intracellular calcium.[35,48] When the ER experiences stress from exposure to chemical toxins, oxidative stress, *etc.*, proteins are misfolded and the ER initiates repair

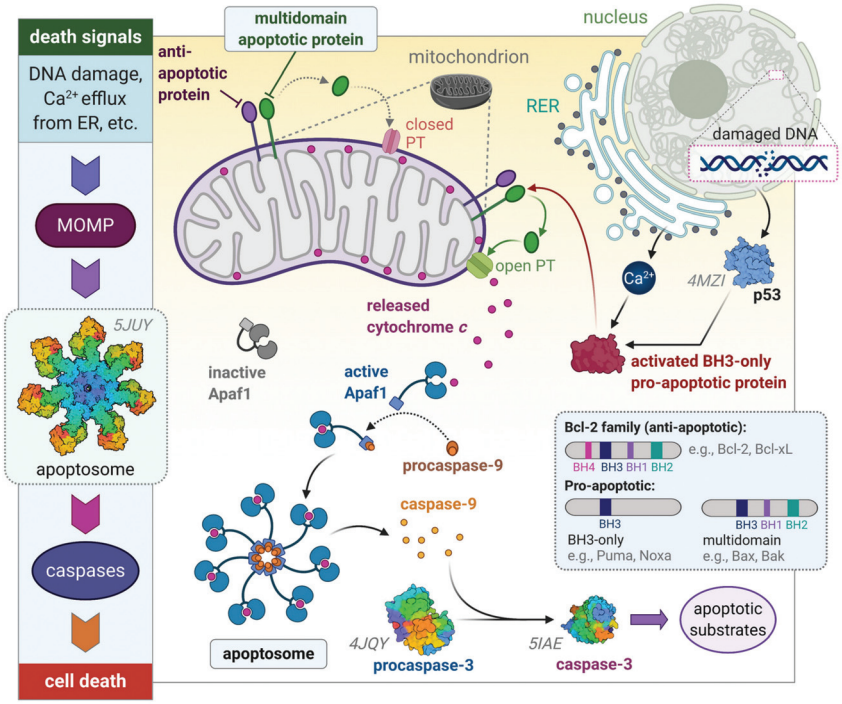


Fig. 3 A basic representation of the intrinsic or mitochondrial apoptotic signalling pathway. Internal signals from the cell converge on the mitochondria, resulting in MOMP mediated by pro-apoptotic proteins of the Bcl-2 family. MOMP results in the release of cytochrome *c*, which binds to Apaf-1 to form an apoptosome with procaspase-9, which is subsequently activated to caspase 9. The effector caspase, procaspase-3, is activated by caspase-9 and activates proteins that execute apoptosis. The insert illustrates how the members of the Bcl-2 family are group based on their functions (*i.e.*, anti- and pro-apoptotic) and number of BH domains. Acronyms: Apaf-1, apoptosis protease-activating factor-1; MOMP, mitochondrial outer membrane permeabilization; PT, permeability transition pore. Protein structures are rendered from their protein data bank (PDB) codes: apoptosome (5JUY), p53 (4MZI), procaspase-3 (4JQY), and caspase-3 (5IAE; the monomeric V266F mutant has been shown for simplicity).

mechanisms to either remedy or degrade the faulty proteins. When the response fails to resolve the problem, the ER releases calcium into the cytoplasm.[47,61] The mitochondria respond by releasing a small amount of cytochrome *c*, where it interacts with ER receptors. The ER releases more calcium, resulting in a mass release of cytochrome *c* from the mitochondria, thereby initiating stress-induced apoptosis.[62,63]

Cancer cells have various mechanisms to avoid apoptosis. In B-cell lymphoma, the anti-apoptotic protein, Bcl-2 is overexpressed, thereby tipping the scales in favour of survival. The loss of p53 is one of the most successful ways cancer cells avoid cell death, as p53 initiates the apoptotic caspase cascade not only in response to DNA damage but also under abnormal conditions such as hypoxia and overexpression of oncogenes (*e.g.*, Bcl-2).[64–66]

Other mechanisms of cell death include necrosis and autophagy.[67,68] Cell death by necrosis is often associated with cells that suffer excessive damage. Necrotic conditions are favoured when normal physiological function (*e.g.*, ion transport, pH balance and cellular respiration) is inhibited due to either pathological events (*e.g.*, microbial or viral infection) or injury. Necrosis is characterized by the release of cellular contents into surrounding tissues due to the swelling of the cytoplasm and the breakdown of the cell membrane, thereby eliciting an inflammatory response by the immune system.[51,53,68]

Cell death can also occur through autophagy,[67,69,70] an intracellular lysosome-mediated catabolic mechanism. Autophagy recovers nutrients such as amino acids during periods of nutrient scarcity by degrading proteins within the cell and digesting damaged or dysfunctional cellular components to ensure cytoplasmic homeostasis. At first glance, autophagy promotes cell survival, but cell death can occur due to extensive vacuolization of the cytoplasm, which engenders cell destruction.[51,53,71]

The relationship between cancer and cell death is unfortunately not straightforward, with apoptosis being able to promote tumourigenesis.[72] Furthermore, cancer cells can initiate autophagy under the same stressful conditions (starvation, oxidative stress, *etc.*) as healthy cells, to the extent that this state becomes protective and reversable, especially when exposed to chemotherapy.[73] In the event of necrotic death, pro-inflammatory signals are elicited, alerting immune inflammatory cells to the site of damage. Bioactive molecules such as growth factors, survival factors and enzymes that promote angiogenesis, invasion, and metastasis are thereby supplied to the tumour microenvironment as a result. In early tumour development, the sacrificial death of a pre-malignant cell possibly allows the development of more aggressive- and apoptosis-resistant cancer cells.[32,42]

This evidence aside, cancer cells avoid death and apoptosis is still the core intended outcome of chemotherapy. However,

considering the tumour environment, drugs that elicit inflammatory responses and unnecessary tissue damage are best avoided. Drugs that are highly effective at killing all tumour cells are ideal as death induced by chemotherapeutic agents imposes a selective pressure on cancer cells. The chance of a treatment-resistant cancer arising increases markedly when low efficacy anticancer agents are used,[42] which is one reason why multi-drug chemotherapy regimens are favoured for certain malignancies.[74]

Cancer is the result of defects in cellular regulatory mechanisms and the examples discussed above do not do the complexity around the disease justice. The list of possible faults in the internal circuitry and potential checkpoint fails is virtually infinite. The advances made in elucidating the molecular biology of distinct cancer types allow for the development of target-specific treatments.[75,76] Unfortunately, many cancer cell targets match those operating in healthy cells; achieving selective chemotherapy thus remains elusive. Ideally, chemotherapeutic agents should have a multi-faceted approach[77,78] to successfully induce apoptosis in cancer cells, a demise such cells dutifully attempt to avoid.

DNA as a cancer drug target

The chemotherapeutic effects of mustard gas, ironically discovered during the First World War, is attributed to its ability to alkylate DNA. The use of mustard gas and nitrogen mustard, an easier-to-manage alternative in clinical trials, represents one of the first examples of DNA-targeted chemotherapy.[17,79] The appropriateness of DNA as an anticancer drug target is validated by the dominant role it plays in mitosis (cell division), the transmission of genetic material, the control of cellular processes (gene transcription), and how closely these functions relate to the development of cancer.[80] However, DNA can be a risky target for anticancer drugs. Topoisomerase II mediated DNA cleavage in the presence of etoposide, for instance, causes chromosomal translocations and acute myeloid leukaemias (AMLs) in 2–3% of patients.[81] Problematically, strategies used to develop compounds that selectively target tumour cell DNA[82] have not been entirely effective.[83] Furthermore, because of their inherent lack of selectivity (*vide infra*), many clinically relevant DNA-targeting drugs extensively damage normal cells such as those constituting bone marrow,[84,85] causing Grade 4 [86] (*i.e.*, life threatening) adverse effects.

The structure of DNA

DNA is a polynucleotide, where each nucleotide consists of a D-2-deoxyribose sugar, a phosphate group, and an aromatic nitrogenous base, of which there are four types: adenine (A), guanine (G), thymine (T) and cytosine (C). The individual nucleotide residues are joined by a phosphodiester bond between the sugar of one nucleotide and the phosphate group of another, forming one DNA strand. The direction of the DNA strand depends on the direction of the phosphodiester bond *i.e.*, a 3′–5′ phosphodiester bond is between carbon-3 of the sugar of one nucleotide and carbon-5 of the sugar of the following nucleotide. The structure of duplex DNA, first presented in 1953 by Watson and Crick,[87] takes on the form of a double helix, whereby two DNA strands are aligned antiparallel (5′–3′ and 3′–5′) and are held together by hydrogen bonds between complementary base pairs (A–T, 2 H-bonds; G–C, 3 H-bonds), as illustrated in Fig. 4a. The base pairs (bp) are stacked in the centre of the helix and the sugar-phosphate backbone strands frame the outside of the helix.[88] Within the eukaryotic cell nucleus, the DNA is wrapped around histone proteins prior to further packaging to form chromatin.[89,90]

The helical structure of B-DNA, the most common conformer of DNA (the others being A- and Z-form), is presented in Fig. 4a. The helix is right-handed and is defined by a helix pitch of 35.7 Å and 10.5 base pairs per turn. Since each helical turn is 360°, each base is rotated 34.3° from the subsequent base. These structural parameters of B-DNA change dramatically (twice the curvature) when DNA is wrapped around the histone protein octamer within the nucleosome core particle.[91] As revealed by an analysis of numerous X-ray structures of DNA and DNA–protein complexes, small local variations in the ideal geometry of B-form DNA induce a wide range of global geometries that accommodate DNA-binding protein motifs.[92] In short, B-form DNA is conformationally flexible. A-form DNA deviates from the B-DNA parameters because of sugar puckering that results in a smaller helix pitch (28.15 Å), 11 base pairs per turn and a significant base tilt of 20° from the helical axis.[93] Another variant, Z-DNA, is a left-handed helix with a zig-zag backbone.[80,88] Of the conformations, the helical structure of B-DNA offers the best distinction between the major and the minor grooves. These grooves vary quite significantly due to the exposure of different nucleobase functional groups as potential binding sites for proteins, small molecules and drugs.[80,88,94]

Interactions of clinically approved anticancer drugs with DNA

Alkylating agents such as nitrogen mustard ($N(CH_3)(CH_2CH_2Cl)_2$) are among the most effective anticancer drugs due to their ability to crosslink DNA strands.[95,96] Two nucleotide residues on the same strand (intrastrand) or opposite strands (interstrand) can be covalently connected by dialkylating agents. In the case of nitrogen mustard, interstrand crosslinks are formed, subsequently preventing the separation of DNA strands during critical cellular processes such as replication and gene transcription. Therefore, nitrogen mustard and its derivatives are most effective against highly proliferating cancers such as leukaemias and lymphomas. Unfortunately, cells in rapidly proliferating healthy tissues are also targeted and this results in side effects such as nausea, vomiting, hair loss and bone marrow toxicity (myelosuppression).[17,79,97]

Other clinically-approved drugs, including cisplatin,[98] mitomycin C[99] and its primary metabolite 2,7-DAM (2,7-diaminomitosene),[100] as well as the natural product trabectedin (Et-743),[101] also covalently bind to DNA (Fig. 4b).[102] Each drug elicits a markedly different cellular response and therefore has

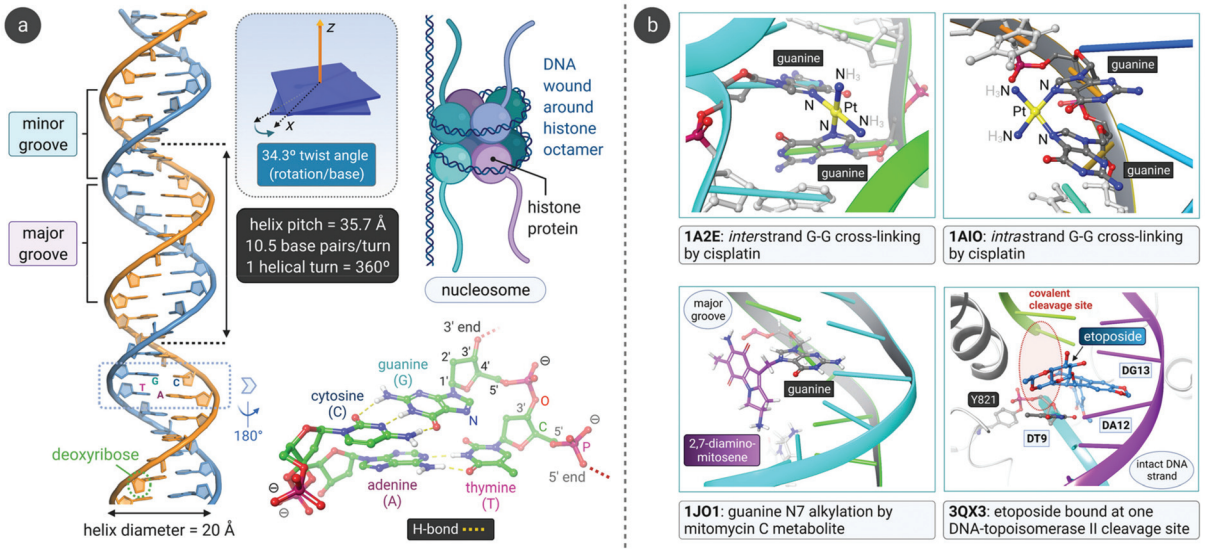


Fig. 4 (a) Double-helical structure of the most common conformer of DNA, B-form DNA, with distinct major and minor grooves. The structural parameters (helical pitch of 35.7 Å, 10.5 residues per turn, 34.3° twist angle between residues and a diameter of 20 Å) associated with B-DNA are indicated. The DNA is wound around histone protein octamers prior to further packaging into chromatin in eukaryote cell nuclei (upper right). The structure of the residues in a 3′-GT-5′ tetramer motif within the left DNA duplex is shown (lower right) to highlight the complementary H-bonding between the nucleobase pairs (polar H atoms are shown along with D-2-deoxyribose C-atom numbering). (b) Illustration of some key DNA interactions by clinically relevant anticancer drugs. Top: cisplatin forms both inter- and intrastrand cross-linkages (*via* covalent bonds to N7 of guanine residues), thereby bending the DNA toward the major groove. Bottom: mitomycin C's main metabolite covalently attached to guanine N7 (left) and etoposide intercalated at one of two covalent cleavage sites (3′-TA-5′) created by topoisomerase II (Top2) during its catalytic cycle with a suitable DNA substrate (right). PDB codes are indicated in bold font for each structure.

a distinctive chemotherapeutic mechanism. Often, drugs that bind to DNA influence the interaction of proteins with DNA since DNA is the substrate to which proteins responsible for DNA replication, gene transcription, packaging, recombination, repair, *etc.*, bind.[17]

After aquation,[103] cisplatin preferentially binds to N7 of two guanine residues on the same (*intra*strand)[102] or adjacent[98] strands (Fig. 4b), forming cross-linked covalent G–Pt–G DNA lesions. These crosslinks significantly bend the DNA towards the major groove (Fig. 5),[102] exposing a minor groove binding pocket to DNA-binding proteins, *e.g.*, high-motility group box proteins (HMGB),[104] repair proteins, transcription factors and other damage recognition proteins. Trapped proteins are prevented from carrying out their intended functions or mask the distortion of DNA from the nucleotide excision repair (NER) mechanism,[105] a process that repairs DNA lesions caused by environmental or chemical damage by recognizing, removing, and resynthesizing the damaged region (these activities form part of the DNA damage response, DDR[106,107]). Precise details regarding the progression from initial damage to cell death remain unclear, but several DNA-damage transduction signal pathways (*e.g.*, AKT, c-ABL, MAPK, p53, *etc.*) are thought to be influenced in response to cisplatin-induced damage.[17,108–117]

While cisplatin kinks the DNA toward the major groove, the naturally occurring anticancer drug trabectedin covalently binds within the minor groove of DNA and unveils the major groove pocket for protein binding. Thus, a range of different proteins are targeted including key players in transcription-coupled NER (the repair of genes that are coded into proteins).[117] Et-743, which targets the DNA sequences 5′-TCCA-3′ and 5′-TGCC-3′,[117] and mitomycin C,[118,119] also a minor groove binder, have far greater DNA binding selectivity than normal DNA alkylators due to their sequence specificity.[17]

Small molecule compounds that influence DNA–protein complexes are not limited to covalent binders. Doxorubicin[120] and etoposide,[121] the former a non-specific DNA intercalator[122] and the latter a non-intercalating compound,[123] are both notably cytotoxic and operate by inducing DNA double strand breaks where the enzyme topoisomerase II (topo II) is covalently bound to its DNA substrate *via* a pair of tyrosine phosphodiester links positioned four bases apart (Fig. 4b).[124] The two drugs act as interfacial poisons of the enzyme by binding noncovalently at the pair of DNA strand nick sites, thereby blocking the enzyme from resealing the transient DNA double strand break to complete its catalytic cycle.[125] Topoisomerases are a family of enzymes which are responsible for maintaining the correct DNA topology during critical cellular functions such as replication and transcription. They introduce temporary single- (topo I) or double-stranded (topo II) DNA breaks.[126] Cancer cells are thought to be more susceptible to topo II poisons than non-tumorigenic cells due to the elevated levels of topoisomerases found in most cancer cells.[17] Interestingly, an investigational cytotoxic Au(III) isoquinoline-amide complex has been shown to act as a dual-mode inhibitor of topo II, poisoning the enzyme at low concentrations (K_D ~240 nM) but catalytically inhibiting it at higher concentrations

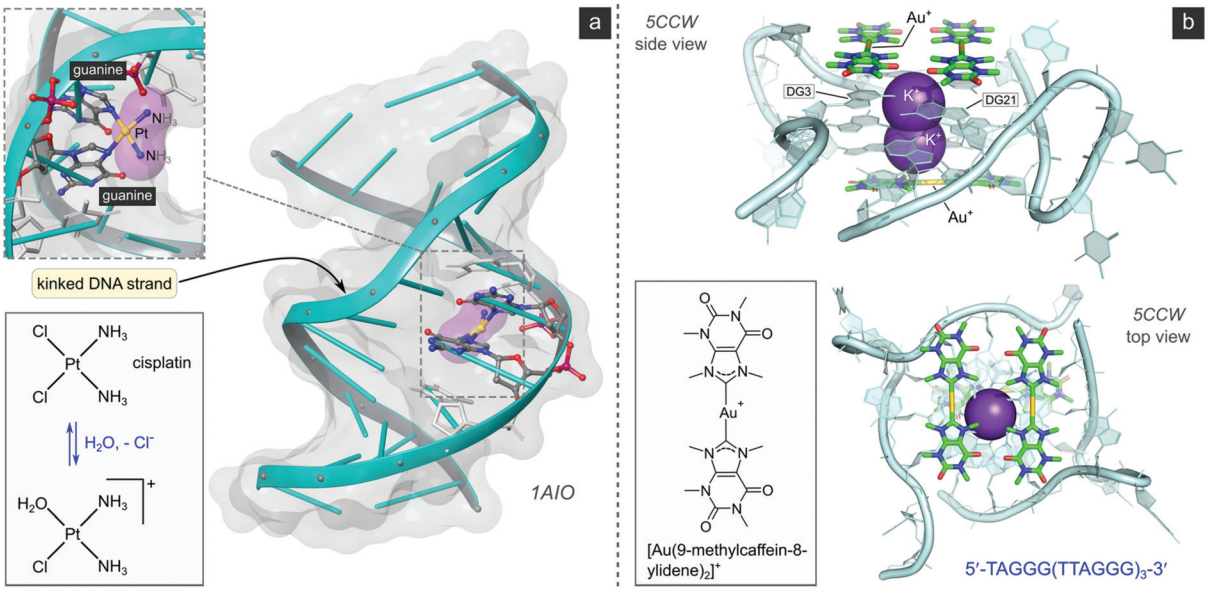


Fig. 5 (a) Illustration of the crystal structure of cisplatin covalently bound to two N7 atoms of adjacent guanine residues. The dsDNA target adopts a distorted helix conformation with a marked kink close to the DNA lesion (PDB 1AIO). Cisplatin itself undergoes aquation to form the mono(aqua) adduct prior to coordination to guanine N7, as depicted in the equilibrium ligand exchange (left side). PDB codes are indicated in italics font for each structure. (b) X-ray structure of the complex formed between a linear Au(I) mesoionic carbene complex and a G-quadruplex DNA target stabilized by centrally located K^+ ions (PDB 5CCW). The planar Au(I) bis(carbene) complexes π-stack atop guanine bases in the central region of the G-quadruplex above and below the cylindrical structure's core.

(K_D ~9 μM).[127] Since the ancillary *cis*-chloride ligands were substitution inert, this class of compounds likely does not covalently bind to DNA (unlike cisplatin), suggesting that future clinically approved topo II-targeting drugs might include metal chelates with unique behaviour.

Although not yet clinically relevant, metallodrug candidates that target DNA secondary structures such as G-quadruplexes are also being investigated.[128] G-Quadruplexes form readily under physiological conditions and consist of intermittent runs of guanine residues. These quadruplex structures mainly form in the promoter (DNA sequences to which the enzyme RNA polymerase binds to initiate protein synthesis) and telomeric regions of the DNA sequence.[129] Telomeres protect the ends of chromosomes during DNA replication and shorten progressively during each cell division until cell death ensues. Cancer cells have highly expressed telomerase activity, the enzyme responsible for the maintenance of telomeres, amounting to another mechanism of acquired immortality. G-quadruplex interacting compounds, which span a range of transition metal complexes,[130] including Au(I) carbenes[131,132] and a polypyridine Ru(II)[133] complex, and which have recently been crystallized with different binding site preferences to G-quadruplex DNA targets (*e.g.*, Fig. 5b), can inhibit telomerase by blocking enzyme-DNA binding or by preventing the formation of G-quadruplexes (*i.e.*, telomeres and promoters) altogether.[17,31,32,35,134] Other DNA secondary structures such as DNA three-way junctions (3WJs) and DNA four-way junctions (4WJs) have also emerged as suitable targets for several investigational organic compounds and metal chelates,[135] as discussed by Zell *et al.* in their excellent review.[136] Notably, metallopeptides[137] and metallocages[138] feature among the more active compounds for targeting 3WJs, benefitting from the ability of the metal ions to assemble the ligands and create the required triangular shape complementarity for the DNA target.[139] Regarding 4WJs, the first metal complex (square planar Pt^{2+} chelated by a tetradentate polypyridyl ligand) that acts as an effective template for the assembly of a 4WJ-like DNA superstructure has been described.[140]

Metal complexes, due to their own high tolerance for structural and electronic modification, can clearly exploit various DNA binding sites with great success.[80,94,134,141] Moreover, metal complexes may interact with a diverse range of molecular targets, not limited to DNA, which might allow the development of metallodrugs with multi-faceted cellular targets. A noteworthy caveat is that such compounds should ideally be designed to accumulate selectively in neoplastic tissue to overcome the limitations of cisplatin[142] and to be realistic future contenders for clinical use. The current surge of investigations into the anticancer potential of metal complexes ultimately stems from the initial discovery of cisplatin's anticancer properties,[143] the delineation of its mechanism of action,[102] and the intrigue that such a simple complex (and structures related to it) has held for chemists, biologists, and pharmaceutical researchers over many decades. The field, however, has a firm foundation and is now poised for significant new discoveries of novel biomolecular targets for metal complexes, as evidenced by the recent report that metallo-supramolecular cylinders which target DNA 3WJs can also target bulge structures in

the 5′ untranslated regions of the RNA genome of SARS-CoV-2 and inhibit viral replication.[144] The emergent picture is that "*DNA* (and RNA[145]) *folds threaten genetic stability and can be leveraged for chemotherapy*",[136] whether the target is cancer or pathogenic viruses. The question is will pharmaceutical firms take promising preclinical metallodrug candidates seriously enough to offer them the same interest, ADMET scrutiny, and development opportunities as seemingly preferred "mono-target" organic compounds? Binding to multiple macromolecular targets, which is often a hallmark (but can also be a serious limitation) of many metallodrug candidates,[146] might be a useful strategy for overcoming drug resistance since multiple simultaneous mutations in unrelated genes would be required by the organism in question to develop resistance to such a drug. This idea is now in vogue[147,148] in the face of the current SARS-CoV-2 pandemic and could become a feasible approach to limit drug resistance in cancer chemotherapy.

Cytotoxic gold complexes

Historic use of medicinal gold

The earliest known application of medicinal gold was in China dating back to 2500 BC in pursuit of longevity; arguably so, as gold itself is resistant to corrosion and rust. In early times, gold powder was rubbed on open wounds and said to remove the toxins associated with smallpox and skin ulcers as well as mercury from the body. Heated gold relieved toothache and golden amulets were worn to repel evil spirits.[149]

The medicinal use of gold was limited to topical ailments as there was no way to dissolve gold. In medieval times, potable gold (*i.e.*, drinkable gold) emerged as elixirs of life to restore youth. These elixirs contained very low concentrations of dissolved gold, as cited in one of the many recipes for potable gold: "to quench a heated gold plate in wine four to five times". Not surprisingly, scepticism arose around the medicinal properties of gold as some potable gold recipes cured ailments (in these recipes *aqua regia* was used to generate the soluble salt, $AuCl_3$) and others did not (*e.g.*, heated gold in wine).[149,150]

In the 17th century, several preparations for medicinal gold were known. One of the preparations required the distillation of dissolved gold and *aqua regia*, resulting in the soluble $AuCl_3$ salt. A second preparation, the fulminate of gold, was made by adding ammonia to *aqua regia* and gold. Not unexpectedly, gold chloride was deemed too corrosive and gold fulminate too dangerous (explosive) for any medicinal use. In the 19th century, after the use of medicinal gold had declined dramatically, a French physician used a less caustic mixture of gold and sodium chloride, a so-called "muriate of gold and soda", to treat syphilis. Although the reproducibility of his findings was ambiguous, by the mid-19th century, this double chloride of gold was prescribed as a secondary drug, only when the primary would fail, to treat morphine addiction, nephritis, anaemia, premature senility, neurasthenia, lupus and alcoholism.[150]

The first rational use of a gold metal complex, $K[Au(CN)_2]$, in medicine is ascribed to Koch in ~1890.[151] The many attributes of metal complexes, such as their variable coordination numbers, geometries, and redox states, in combination with appropriate organic ligands, are particularly useful for drug discovery and design. Metal ions generally target diverse biological molecules depending on the element, type of coordinated ligands, coordination geometry, kinetic lability (ligand exchange), and redox potential. Indeed, the same metal ion in different oxidation states can bind to different drug targets.[152–156]

Gold(I) compounds, for instance, are classified as soft electrophiles in Pearson's HSAB theory[157,158] and interact preferentially with soft sulfur-donor and phosphane ligands (nucleophiles). Because of the ubiquity of cysteine in proteins, Au(I) can bind to solvent-exposed cysteine thiolate groups and subsequently inactivate enzymes, particularly those with catalytic cysteine residues such as the cysteine proteases.[159,160] Hard Au(III) ions favour harder nitrogen and oxygen donor ligands, which is one reason why Au(III) salts were initially used to stain nucleic acids for imaging by electron microscopy.[151] Moreover, the redox chemistry and associated change in coordination number of gold complexes can be an advantage as the reduction of the square-planar Au(III) ion to the linear Au(I) ion is often associated with the release of ligands, whereby these ligands can have inherent medicinal properties.[24] In principle, Au(III) complexes can be used to deliver both a clinically approved drug and Au(I) to a tumour if appropriately designed, akin to Pt(IV) systems.[161,162]

Koch found that $K[Au(CN)_2]$ was bacteriostatic against "tubercle bacillus", a strain of bacteria now known as *Mycobacterium tuberculosis* and the causative agent of tuberculosis.[163] During clinical trials, however, $K[Au(CN)_2]$ ("gold cyanide") proved ineffective in treating tuberculosis and had serious toxic side effects,[151] stemming any further use of the compound. Gold(I)-thiols, such as sodium aurothiomalate (Fig. 6 and 7) were much less toxic and were administered to tuberculosis patients during the period 1925–1935 (known as the Gold Decade) but the usage declined as reports on toxicity

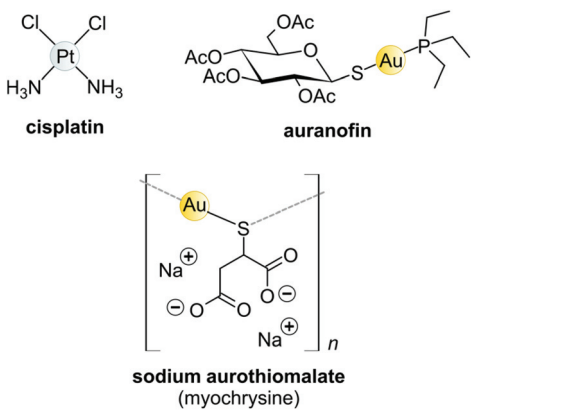


Fig. 6 Structures of selected metal-based chemotherapeutic drugs in clinical use (Pt^{2+} and Au^{+} complexes).

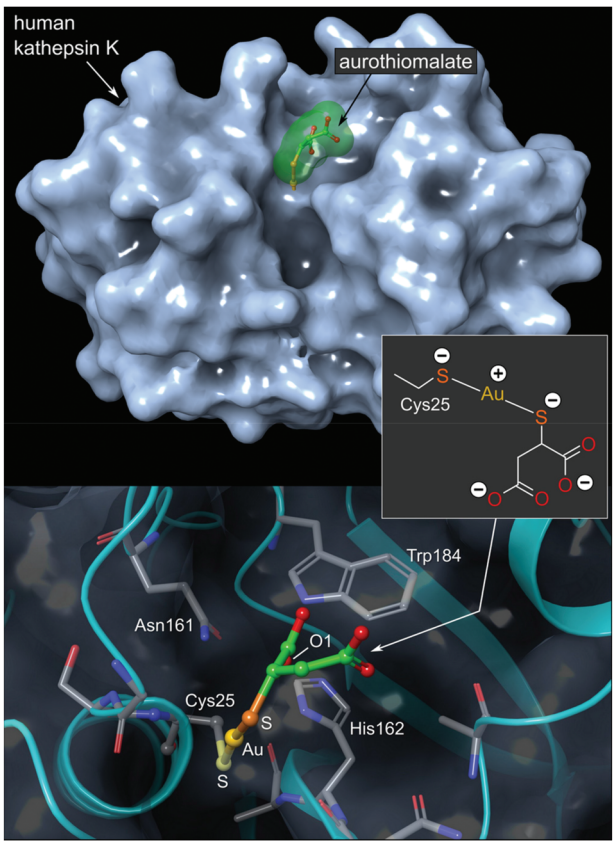


Fig. 7 Top: View of the X-ray structure (PDB 2ATO) of human cathepsin K (a cysteine protease) covalently bound to myochrysine (aurothiomalate). The linear Au(I) thiolate targets Cys25 within the enzyme's active site cleft. Bottom: close-up view of the coordination of the Au(I) ion to the thiolate ligand of Cys25. The S–Au–S bond angle measures 173.1°; the Au–S distances are equivalent at 2.5 Å. The O1 atom of thiomalate is an acceptor for three NH···O hydrogen bonds with Gln19, His162, Trp184 (not shown).

increased along with very little evidence of treatment efficacy. Later on, these gold thiolates were investigated against rheumatoid arthritis after the idea that tubercle bacillus caused rheumatoid arthritis.[150,163,164]

Today, after years of debate and results obtained from a large, well-controlled double-blind trial concluded that gold compounds have a beneficial effect on rheumatoid arthritis (RA),[165] injectable Au(I)-thiolates have been included in a group of FDA-approved disease-modifying antirheumatic drugs (DMARDs).[164,166,167] These drugs do have notable efficacy limitations as they are slow acting and accumulate in the kidneys, giving rise to nephrotoxicity.[163] In the 1970s, an orally administered gold(I)-phosphane drug, auranofin (tetraacetyl-β-D-thioglucose gold(I) triethylphosphine, Fig. 6)[168,169] was introduced and although only mild side-effects were reported, auranofin was less effective than the injectable gold drugs in treating RA.[134,141] However, auranofin is still used clinically today for psoriatic arthritis and juvenile rheumatoid arthritis when other front-line DMARDs like methotrexate[170] fail to treat refractory RA.[164,166,167]

The Au(I) complexes, auranofin and myochrysine (Fig. 6), another approved antiarthritic drug, are currently undergoing clinical trials as anticancer drugs.[171,172] Auranofin is an inhibitor of the enzyme thioredoxin reductase, increases cellular ROS, and induces the mitochondrial apoptotic pathway.[166,167,173] Myochrysine, an aurothiomalate complex, specifically binds to a cysteine residue in the binding pocket of protein kinase C iota (PKCi), preventing the downstream protein, par-6, from binding to the kinase. The PKCí···par-6 complex is a signalling intermediate within a cellular pathway that stimulates tumour growth and invasion.[174,175] Myochrysine covalently bound to the cysteine residue (Cys25) in the active site of human cathepsin K has been structurally elucidated by X-ray crystallography (Fig. 7), representing one of the first gold–protein adducts to be studied at near-atomic resolution. The thiomalate ligand does not dissociate from the Au(I) ion and participates in hydrogen bonding *via* its carboxylate groups with several nearby residues.[176]

Biological targets and antitumour activity of Au(I) compounds

The use of gold(I) compounds as potential anticancer agents was sparked by the *in vitro* cytotoxicity of auranofin against HeLa (human cervical epithelioid carcinoma) cells[177] and further studies that revealed auranofin's similar, or in some cases superior, cytotoxicity compared to cisplatin.[171,178,179] The *in vivo* activity of auranofin, however was found to be limited to leukaemia P388 mouse models only.[180–182] Once injected, auranofin undergoes rapid ligand exchange and binds covalently to the cysteine residue (Cys34) of human serum albumin, the most abundant blood protein,[183] an event which subsequently decreases the concentration of the active gold species and the rate of cellular uptake.[184] The first structural evidence of Cys34 being a gold-binding site was recently reported (Fig. 8). Messori and co-workers co-crystallized BSA (bovine serum albumin) with a dithiocarbamate Au(III) complex. Upon binding to the protein, the Au(III) ion was reduced to Au(I) with concomitant loss of the ligands (2 Br^- and $C_6H_{10}NO_2S_2^-$), resulting in a "naked" Au(I) ion bound to Cys34 of BSA.[185] Because of the low resolution of the structure, the identity of the second ligand (probably water) was not established.

Despite limited *in vivo* activity, auranofin is a potent inhibitor of mitochondrial thioredoxin reductase (TrxR2), elevating this enzyme's status to that of a promising new drug target for investigational Au(I) complexes,[173,186] but perhaps realistically only if compounds can be designed to selectively target tumour cells (since TrxR2, though not overexpressed as in tumour cells,[187] is present in the mitochondria of all human cells which utilize this organelle[188]). Thioredoxin reductases (TrxR) are a class of enzymes that catalyse the reduction of thioredoxins (Trx), proteins which themselves are responsible for maintaining other cellular proteins in their reduced state. TrxR, Trx and the redox co-enzyme, NADP(H) collectively form the Trx system, a ubiquitous arrangement that is crucial in maintaining intracellular redox conditions (Fig. 9). Reduced Trx is responsible for the reduction of cellular proteins that

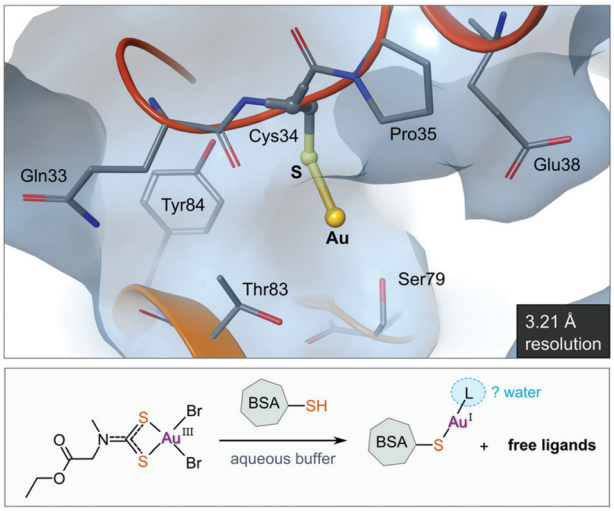


Fig. 8 View of the X-ray structure of the Au(I) adduct of bovine serum albumin (BSA) formed after incubation of the protein with the Au(III) complex of (2-ethoxy-2-oxoethyl)methylcarbamodithioate (PDB 6RJV). Reduction of the Au(III) complex occurs with concomitant release of the ligands to form the linear Au(I) adduct with Cys34 thiolate (Au–S, 2.14 Å). Water molecules were not located in the low-resolution X-ray structure, but it is possible that a second ligand (water) completes the coordination sphere of the linear Au(I) ion.

play critical roles in cell division, transcription of genes, apoptosis and proteins that protect the cell against ROS species. Specifically, Trx reduces proteins such as ribonucleotide reductase, a critical enzyme in DNA synthesis, and thioredoxin peroxidase which catalyses the reduction of hydrogen peroxide to water.[189–191]

The Au(I) ion of auranofin binds covalently to the selenocysteine (Sec, U) residue in the active site of TrxR with impressive selectivity since the drug binds to the mechanically and structurally similar, but selenium-free enzyme glutathione reductase (GR) at a 1000-fold higher concentration.[173,192] Au(I) binding prevents the substrate, Trx, from binding and undergoing subsequent reduction by TrxR. The mitochondrial PT pores (Fig. 3) sense the imbalance of thiol proteins, open and induce MOMP, which ultimately leads to cell death due to the release of cytochrome *c*. MOMP is also induced by the accumulation of H_2O_2, as auranofin prevents the removal of hydrogen peroxide by inhibiting TrxR. Furthermore, MOMP leads to the leaking of important respiratory chain components into the cytosol, inevitably causing the respiration chain to uncouple and the production of ATP to cease. Indeed, a decrease in basal oxygen consumption and ATP output of cells has been observed in auranofin-treated cells.[52,173,187]

The mitochondrion is, in principle, a suitable drug target for chemotherapeutics that can be selectively taken up by tumour cells as this organelle is a crucial regulator of cell death, an endpoint most cancer cells are resistant to. These resistance pathways, due to either defective pro-apoptotic or overexpressed antiapoptotic members, are potentially avoided with compounds that induce MOMP directly.[193,194] The challenge, however is to target the mitochondria of cancer cells selectively since mitochondria are present in most human cells. The selective inhibition of mitochondrial TrxR is a strategy which exploits the fact that cancer cells have elevated levels of these proteins to protect themselves from the high levels of ROS generated due to increased proliferation and respiration.[187] When these proteins are inhibited, cancer cells are much more vulnerable to these elevated intracellular ROS levels than healthy cells.[189,191] Unfortunately, most known TrxR inhibitors cannot distinguish between mitochondrial TrxR and the cytosolic TrxR, and MOMP is only associated with the effective inhibition of mitochondrial TrxR.[173,187]

Another approach to targeting the mitochondria of cancer cells is the development of a diverse group of compounds

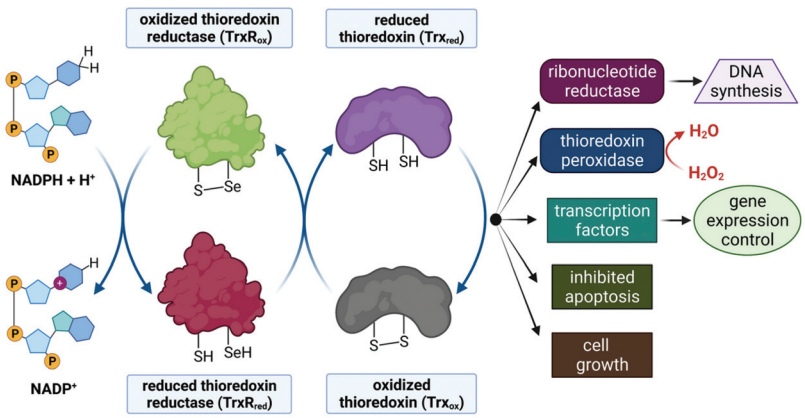


Fig. 9 Schematic illustration of thiol-disulphide exchange in the Trx system. Left: the co-enzyme NADPH is oxidized to NADP$^+$ to reduce TrxR$_{ox}$ (–S–Se–) to TrxR$_{red}$ (–SH, –SeH), which subsequently reduces Trx$_{ox}$ (–S–S–) to Trx$_{red}$ (–SH, –SH), as depicted in the centre two cycles. Trx$_{red}$ is then available to reduce substrate proteins or to carry out required cellular functions (right side). Thus Trx reduces ribonucleotide reductase, an enzyme that reduces ribose to deoxyribose (the sugar of DNA nucleotides required for DNA synthesis). Trx also reduces other proteins such as thioredoxin peroxidase, which is responsible for converting toxic H_2O_2 to water as well as transcription factors that regulate gene expression. In addition, Trx functions as a cell growth factor and can inhibit apoptosis.

called delocalized lipophilic cations (DLCs).[195] These compounds, which include metal chelates,[196] are characterized by elevated partition coefficient values to readily pass lipid membranes and a delocalized positive charge,[197] which allows them to be driven towards negatively charged species. In fact, the mitochondria of cancer cells have elevated mitochondrial membrane potential ($\Delta\psi_m$) due to an increased rate of ATP production. Hyperpolarized mitochondria are linked to more aggressive and treatment-resistant cancers. DLCs selectively accumulate in the mitochondria of cancer cells due to the difference of $\Delta\psi_m$ between healthy and cancer cells.[173,187]

The DLCs reported by the group of Berners-Price such as the tetrahedral Au(I) phosphane complex **3** (Fig. 10)[198,199] and the cationic bis-N-heterocyclic (NHC) carbene Au(I) complex **4** (Fig. 10)[200,201] showed high selectivity towards breast cancer cell lines. These findings were not due to chance but a result of carefully considered lipophilic characteristics that are required for the selective accumulation of DLCs in cancer cells.[202,203]

The parent compound of **3**, the tetrahedral Au(I) phosphane complex **1** (Fig. 10), was developed as an attempt to decrease the known tendency of linear two-coordinate Au(I) complexes to rapidly undergo ligand exchange reactions under physiological conditions. They are very reactive towards thiol proteins in blood serum, limiting their *in vivo* cytotoxicity, such as the case with auranofin.[181,204] Complex **1** is stable against GSH (reduced glutathione), a thiol-containing tripeptide used as a model protein to assess the reactivity of Au(I) complexes towards either blood thiol proteins such albumin or the potential of inhibiting a thiol-containing protein target such as TrxR. Although complex **1** exhibited excellent *in vitro* and *in vivo* activity,[205] it was found to be very toxic during preclinical trials, likely due to the high lipophilicity of the complex as non-selective accumulation in the mitochondria leads to severe mitochondrial dysfunction. Moreover, the extreme stability of **1** towards GSH suggests that TrxR would be an unlikely intracellular target.[167,173]

The first-generation tetrahedral phosphane complexes (type 1, Fig. 10) were redeveloped to assess the influence of the lipophilic/hydrophilic ratio on *in vitro* and *in vivo* anticancer activity. By substituting the phenyl substituents with more hydrophilic 2-, 3- or 4-pyridyl moieties (salts of type **2**), a range of complexes could be assessed with varying degrees of lipophilic and hydrophilic character. The complexes with higher lipophilic character were more cytotoxic, but also less selective. The more hydrophilic complexes were significantly less cytotoxic, due to low cellular uptake, high rates of excretion, and low protein binding affinity. From these results, the complex with intermediate lipophilicity, **2**, was the best performing complex, in both *in vitro* and *in vivo* studies.[167,173,206]

The hydrophilic/lipophilic balance was further fine-tuned for the second-generation complexes, where the ethyl linker of **2** was replaced with a propyl bridge (**3**, Fig. 10). By increasing the chelate ring size, ligand exchange reactions with thiol/selenol proteins are more favourable. Complex **3** therefore possesses lipophilic properties resembling those of the first-generation complex **2**, but potentially has auranofin's protein inhibiting properties due to improved ligand exchange.[167] Complex **3** is highly selective towards breast cancer cells and mechanistic studies reveal that **3** selectively accumulates in the mitochondria and inhibits TxrR.[199]

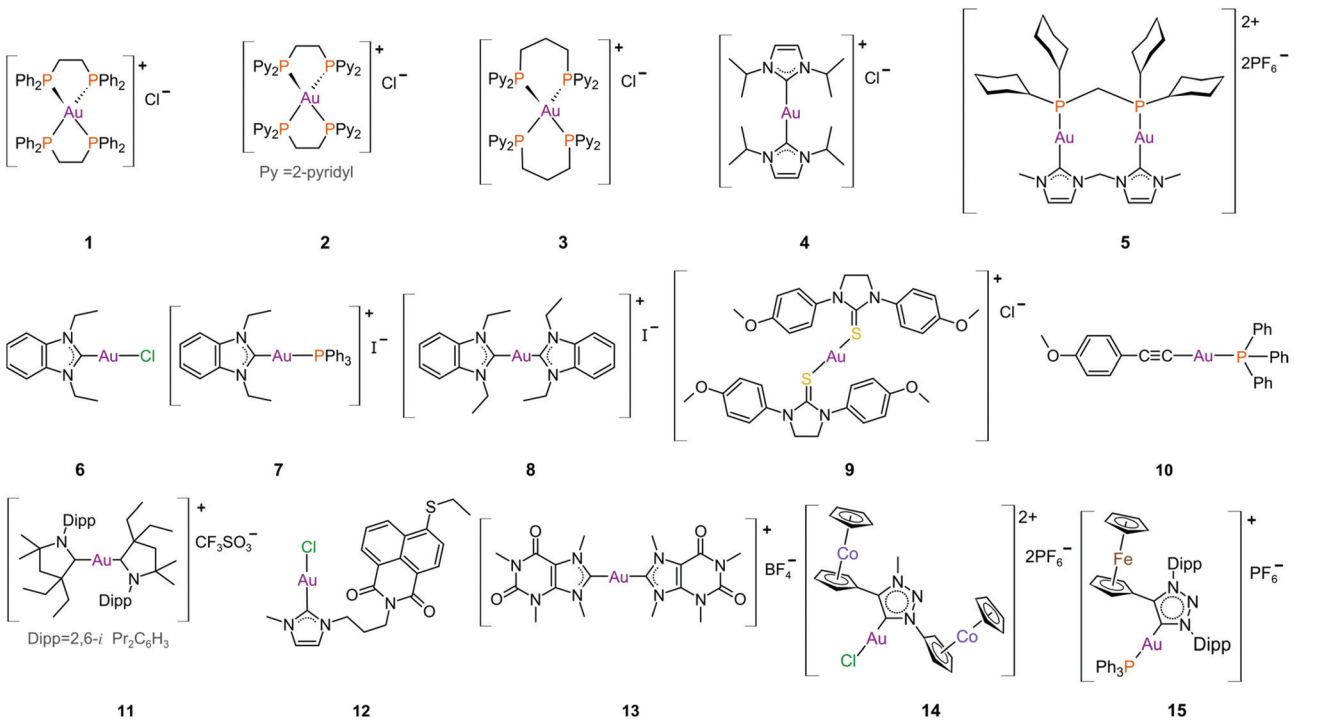


Fig. 10 Structures of selected cationic and neutral gold(I) complexes with promising anticancer activity.

From the same research group, the cationic bis(NHC) Au(I) complexes of type **4** (Fig. 10) were also reported to be selective towards breast cancer cells.[201] Imidazol-2-ylidene-based NHCs are a very attractive class of ligands mostly because a wide range of metal complexes can be synthesized from their precursor imidazolium salts.[186,207–215] NHCs have captured significant attention over the last few decades, predominantly as highly successful ancillary ligands to transition metals in catalytic processes.[216–220] This popularity is warranted as NHCs are easy to synthesize, exceptionally stable, and are amenable to both structural and electronic modulation. Further, the metal–carbene bond is strong, which underpins the traction that NHC metal complexes have in chemical biology as promising anticancer and antimicrobial agents.[221–226] Gold(I) bis(NHC) complexes of type **4** also accumulate in the mitochondria and trigger mitochondrial swelling.[200] Kinetic studies on these complexes show that the substitution of the NHC ligands is faster for selenium-containing residues (Sec) than for thiols (Cys) thereby offering an explanation for the high, selective inhibition of TrxR over GR. Bulkier NHC *N*-substituents (*e.g.*, isopropyl in complex **4**) induce a slower reactivity towards the protein, most likely due to the added steric protection preventing the substitution with Cys/Sec residues.[201]

Combining the properties of both phosphanes and NHCs, Che's group reported on the anticancer properties of a dinuclear Au(I) complex with a bridging diphosphane and a bridging bis(NHC) ligand (**5**, Fig. 10).[227] The complex displayed sufficient stability against serum proteins and adequate reactivity towards thiol proteins, which culminated in a potent TrxR inhibitor (0.038 μM, entry 1, Table 1). This complex is the first reported Au(I) derivative to inhibit cancer stem cell activity and inhibit angiogenesis *in vivo*.[24,184]

The benzimidazole NHC complexes reported by Ott *et al.*,[228] although not very selective in terms of cytotoxicity, contributed valuable information regarding the anticancer properties for this class of NHCs. For instance, the cationic complexes (**7** and **8**, Fig. 10) were more cytotoxic than the neutral complex **6**, with higher cellular uptake and increased accumulation in the mitochondria. However, the neutral complex, due to the more labile *trans* chloride ligand, was the best and most selective inhibitor of TrxR (24-fold over GR) in this series.[228–230]

Gold(I) adducts of other ligands such as thiourea (**9**, Fig. 10) and alkynes, **10**, are notably potent TrxR inhibitors. In fact, complex **9** is the most potent complex ever reported with a K_i (inhibitory dissociation constant) value ~18 to 36 pM (entry 6, Table 1).[231] Auranofin by comparison has a K_i value of 4 nM (entry 18, Table 1).[192] However, the cytotoxicity of both cisplatin (entry 13, Table 1) and auranofin (entry 17, Table 1) against HeLa cells surpass that of **9**. Alkynes like **10** are also potent TrxR inhibitors (entry 7, Table 1) with very promising anticancer metallodrug potential.[232] In contrast, the CAAC (cyclic (alkyl)(amino)carbene) gold(I) DLCs of type **11** (Fig. 10) did not inhibit TxrR and were unreactive towards to BSA. This was attributed to very strong metal–carbene bonds involving the CAAC ligands. Interestingly, the most cytotoxic complex (**11**, Fig. 10), which transcended that of auranofin (entry 8, Table 1), was the least lipophilic of the series examined and exhibited the highest cellular uptake. However, no mitochondrial localization occurred in the presence of **11**. Therefore, the cytotoxicity of **11** is likely cell-based, but independent of the mitochondria or the inhibition of related proteins.[233]

To enhance the cytotoxicity of Au(I) complexes, some Au(I) NHC derivatives have been synthesized with macromolecule-

Table 1 The cytotoxicity (IC_{50}) of the gold(I) complexes **5–15** against cancer- and non-cancer cells. For comparison, the IC_{50} values of cisplatin and auranofin are also shown. Where applicable, the inhibition of the enzyme thioredoxin reductase (TrxR) is also reported

Entry	Complex	IC_{50}[a] (μM): cancer cells (cell line, incubation time)	IC_{50}[a] (μM): non-cancer cells (cell line, incubation time)	IC_{50}[a] (μM): isolated TrxR
1	**5**	1.8 ± 0.6 (HeLa, 72 h)[b]		0.038 (TxrR1, rat)
2		2.5 ± 0.4 (MCF-7, 72 h)[b]		
3	**6**	4.6 ± 0.03 (MCF-7, 96 h)[c]	10.3 ± 1.1 (HEK293, 72 h)[c]	0.4 ± 0.04 (TxrR, rat)
4	**7**	0.9 ± 0.4 (MCF-7, 96 h)[c]	0.4 ± 0.2 (HEK293, 72 h)[c]	0.7 ± 0.02 (TxrR, rat)
5	**8**	0.8 ± 0.1 (MCF-7, 96 h)[c]	3.1 ± 0.5 (HEK293, 72 h)[c]	4.9 ± 1.15 (TxrR, rat)
6	**9**	14.6 ± 0.7 (HeLa, 72 h)[b]		K_i = 18–36 pM (TxrR, rat)
7	**10**	1.0 ± 0.2 (MCF-7, 96 h)[c]		0.05 ± 0.03 (TxrR, rat)
8	**11**	0.3 ± 0.2 (HeLa, 96 h)[d]		>1 (TxrR, rat)
9	**12**	2.1 ± 0.5 (MCF-7, 96 h)[c]		0.4 ± 0.1 (TxrR, rat)
10	**13**	16.2 ± 2.1 (A2780, 72 h)[b]	>100 (HEK293T, 72 h)[b]	
11	**14**	49.6 ± 4.2 (MDA-MB-231, 72 h)[c]	34.3 ± 6.7 (RC-124, 72 h)[c]	
12	**15**	0.2 ± 0.1 (H1975, 48 h)[b]	5.4 ± 0.3 (HEK293, 48 h)[b]	
13	**Cisplatin**	4.7 ± 0.3 (HeLa, 72 h)[b]	11.0 ± 2.9 (HEK293T, 72 h)[b]	
14		5.2 ± 1.9 (A2780, 72 h)[b]		
15		33.7 ± 7.8 (MCF-7, 72 h)[b]		
16		7.8 ± 0.8 (MDA-MB-231, 72 h)[c]		
17	**Auranofin**	1.8 ± 0.1 (HeLa, 72 h)[b]	1.7 ± 0.3 (HEK293T, 72 h)[b]	0.02 (TxrR, human)
18		1.2 ± 0.5 (A2780, 72 h)[b]		K_i = 4 nM
19		1.0 ± 0.3 (MCF-7, 72 h)[b]		0.009 ± 0.000 (TxrR, rat)

Cell viability data were reported as [a] the half maximal inhibitory concentration (μM) required to inhibit the growth of 50% of cells in the culture or 50% of the enzyme activity (IC_{50} values) and were determined by either [b] the MTT assay (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), [c] crystal violet assay, or [d] the cell titre assay.

targeting substituents, such as the DNA intercalator naphthalimide (**12**, Fig. 10), to garner a multi-faceted cytotoxic response. Indeed, complex **12** functions as both a TxrR inhibitor and a DNA intercalator.[234]

Taking advantage of the imidazole ring of caffeine, Casini *et al.* developed a series of symmetric bis(carbene) complexes of type **13** (Fig. 10).[235] Even though the cytotoxicity of **13** against A2780 cancer cells (entry 10, Table 1) was somewhat poorer than that of both cisplatin (entry 14, Table 1) and auranofin (entry 18, Table 1), its cytotoxicity towards human embryonic kidney HEK-293T cells (non-tumorigenic) was practically negligible (IC_{50} >100 μM), signalling great specificity towards the investigated tumour cell lines.[235] Impressively, complex **13** selectively interacts with DNA G-quadruplex structures in a non-covalent manner by π-stacking atop guanine bases between stacks of telomeric sequences (Fig. 5b).[131,235] Following extensive cell-based approaches and shotgun proteomic studies, it was revealed that **13** can enter the nuclei of A2780 cells and bind to DNA. The mechanism of action is considered multi-modal, not only inducing stress responses, but also interfering with the expression of nuclear and telomeric proteins.[236]

After the first report of Ru(II) and Os(II) NHC complexes based on 1,2,3-triazolylidene ligands (trz) detailing potential anticancer properties and high selectivity towards cancerous cells,[237] the cytotoxicity of bimetallic gold(I) trz complexes **14** and **15** (Fig. 10) was investigated. Complex **14** [238] was moderately cytotoxic towards several breast and colon cancer cell lines (entry 11, Table 1). The complex was, unfortunately, more cytotoxic towards the non-tumorigenic RC-124 kidney cell line (entry 11, Table 1). The monocationic Au(I) complex **15** showed antiproliferative activity against the lung cancer cell lines A549 and H1975 in the sub micromolar range (IC_{50}, 0.2–0.9 μM, entry 12, Table 1) with a rather limited selectivity ratio relative to the non-tumorigenic human embryonic kidney cell line HEK-293 (IC_{50} ~5 μM).[239]

In comparison to the salts of conventional NHCs (*i.e.*, imidazol-2-ylidenes) trz salts are more σ-donating as the removal of one σ-withdrawing nitrogen adjacent to the carbene increases the basicity of the coordinated carbene.[240–243] Further fine-tuning of the required properties can be accomplished by altering the wingtip- and C5-substituents of the heterocycle. Synthetic modifications are inherently tractable thanks to the high functional group tolerance of the CuAAC reaction (CuAAC = Cu^{I}-catalysed azide–alkyne cycloaddition) employed for the synthesis of the precursors. Since the first report, trz complexes have been successfully incorporated as suitable pre-catalysts for a number of organic transformations and explored for their photophysical properties.[244–247] In the realm of bioinorganic chemistry, however, the use of trz derivatives as ligands remains underexplored.

Many more Au(I) complexes bearing structurally diverse ligands have been reported that show promising *in vitro* and *in vivo* cytotoxicity. The most probable biomolecular target(s) for binding Au(I) complexes are proteins with readily deprotonated cysteine (–SH) and selenocysteine (–SeH) residues. Covalent adduct formation involving these residues results in outright inhibition or structural changes in the protein which, in turn, may elicit mitochondrial changes and thence apoptosis. Gold(I) complexes do undergo ligand exchange and careful selection of the ligands[248] can dictate the reactivity of Au(I) species with thiol proteins, which can subsequently lead to either their selective targeting of cellular proteins or inactivation by serum proteins.[249–253] The other biologically relevant oxidation state of gold, namely Au(III), has recently re-emerged as a class of compounds with potent cytotoxicity against cancer cells.[254–258]

Biological targets and antitumour activity of Au(III) compounds

Gold(III) complexes were among the first complexes investigated as alternatives to platinum drugs and justifiably so, as Au(III) and Pt(II) are both isoelectronic (d^8), square planar complexes[259–261] with a high binding affinity for DNA nucleotides.[151,262] However, the kinetic lability, light sensitivity and the tendency for the Au(III) ion to reduce to Au(I) or Au(0) under physiological conditions hampered investigations considerably until some Au(III) complexes, years later, were reported with improved stability and antitumour activity.[253,263]

Regarding the earlier literature, of the most noteworthy were the complexes reported by Buckley *et al.* where **16** (Fig. 11) was stable against reduction in the presence of GSH and underwent hydrolysis in water to form the bis(aqua) species [Au(damp)$(H_2O)_2$] (damp = 2-[(dimethylamino)methyl] phenyl), similar to cisplatin's reactivity in biological medium. Both the *in vitro* (entry 1, Table 2) and *in vivo* cytotoxicity showed promising but modest results compared to cisplatin.[23,264–266] During the same period, the monodentate (**17**, Fig. 11) and bidentate (**18**) pyridine-based Au(III) complexes were reported by Messori *et al.*; however, the complexes underwent rapid ligand exchange in water. Despite showing promising *in vitro* cytotoxicity, especially against cisplatin resistant cell lines (entries 2 and 3, Table 2), these complexes were deemed too unstable for medicinal purposes and were not pursued further.[267] Polydentate N-donor ligands considerably improved the stability of Au(III) complexes in solution and soon numerous complexes bearing these ligands were reported. However, reduction to Au(I) in the presence of ascorbic acid or GSH occurs more readily with N-donor functionalities followed by the release of coordinated ligands.[24,184,255,268,269] For instance, the cytotoxicity of complexes **19** and **20** (Fig. 12), although very promising (entries 4 and 5, Table 2), was close to the cytotoxicity reported for the

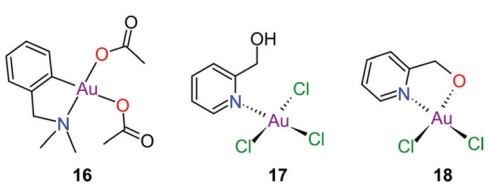


Fig. 11 Early examples of Au(III) complexes evaluated for their antitumour activity.

Table 2 Cytotoxicity (IC_{50}, μM) of the Au(III) complexes **16–21** and **27** with human ovarian carcinoma cell line A2780 and the cisplatin-resistant equivalent cell line, A2780R. For comparison, the IC_{50} (μM) values of cisplatin that were measured under the same conditions are shown italicized in brackets

Entry	Complex	Cytotoxicity (IC_{50}/μM)[a,b] A2780[c] cell line	Cytotoxicity (IC_{50}/μM)[a,b] A2780R[d] cell line
1	**16**	3.5, 96 h *(1.2)*	35, 96 h *(10)*
2	**17**	10.0 *(5.28)*	21.0 *(57.0)*
3	**18**	8.4 *(5.28)*	13.8 *(57.0)*
4	**19**	3.8 ± 1.1, 72 h *(1.2 ± 0.4)*	3.5 ± 0.9, 72 h *(14.2 ± 2.7)*
5	**20**	0.1, 72 h *(1.2 ± 0.4)*	0.4, 72 h *(14.2 ± 2.7)*
6	**21**	1.8 ± 0.2, 72 h *(2.1 ± 0.1)*	4.8 ± 0.5, 72 h *(24.4 ± 0.1)*
7	**27**	3.3 ± 1.4, 72 h	8.2 ± 1.5, 72 h

Cell viability data were reported as [a] the half maximal inhibitory concentration (μM) required to inhibit the growth of 50% of cells in the culture (IC_{50} values) during [b] indicated time of drug exposure and were determined by the sulforhodamine assay. [c] Human ovarian carcinoma cell line. [d] Cisplatin-resistant human ovarian carcinoma cell line.

gold-free phenanthroline and terpyridine (terpy) ligands, respectively.[22] It has been suggested that complex **21** (Fig. 12) also undergoes reduction to Au(I) intracellularly, associates with and causes oxidative damage to proteins and DNA.[256,257]

Che's group used the redox chemistry of Au(III) chelated by tridentate N-donor pincer ligands to design a range of complexes (*cf.* **22** and **23**, Fig. 12) that act dually as fluorescent thiol probes and exhibit cytotoxicity. These complexes bear the highly emissive 2,6-bis(imidazol-2-yl)pyridine (**22**) and 2,6-bis(benzimidazol-2-yl)pyridine (**23**) ligands which act as fluorescent probes when the Au(III) ion is reduced to Au(I) by thiol proteins or GSH. Reduction switches on emission (upon excitation) from the metal-free pincer ligand. The released Au(I) ion remains coordinated to the NHC ligand and subsequently inhibits TrxR, therefore being cytotoxic to cancer cells.[268]

The water soluble corrole-based Au(III) complex **24** (Fig. 12) effectively protects the Au(III) ion from reduction and demetallation. Complex **24** exhibited greater cytotoxicity (entry 3, Table 3) compared with the Ga(III) analogue and outperformed cisplatin on most cell lines. The activity is likely attributed to the low binding affinity of **24** towards HSA.[270]

Au(III) porphyrin complexes of type **25** (Fig. 12) are characterized by high redox stability and cannot be reduced by GSH. These complexes are reportedly very successful cytotoxic agents as **25** is significantly more potent *in vitro* than cisplatin (entry 4, Table 3), especially in cisplatin- and multidrug resistant cell lines. Moreover, **25** can inhibit the self-renewal ability of cancer stem-like cells. The molecular target of **25** remains elusive but extensive mechanistic studies suggest that **25** reduces the mitochondrial membrane potential and targets the mitochondrial chaperone Hsp60 (heat shock protein 60), a protein that is involved in the transport and folding of mitochondrial proteins.[24,184,271]

Munro's group[272] reported the effective inhibition of human topoisomerase IB (topo I) with the use of predominantly planar cationic pyrrole-based Au(III) macrocycles (**26**, Fig. 12) and proved that these complexes' mode of action involves highly specific unconventional catalytic inhibition of topo I. Cation **26** intercalates DNA directly at the sequence targeted by the enzyme and therefore blocks binding of topo I to its DNA substrate. Molecular simulations demonstrated the pivotal role of the Au(III) ion in both the intercalation to DNA and enzyme inhibition. The *in vitro* cytotoxicity of **26** was investigated against the NCI's (National Cancer Institute) panel of 60 human cancer cell lines and the average IC_{50} value was 16 ± 7 μM; the most sensitive cancer cell line was non-small lung cancer, for which an IC_{50} of 280 nM was obtained.

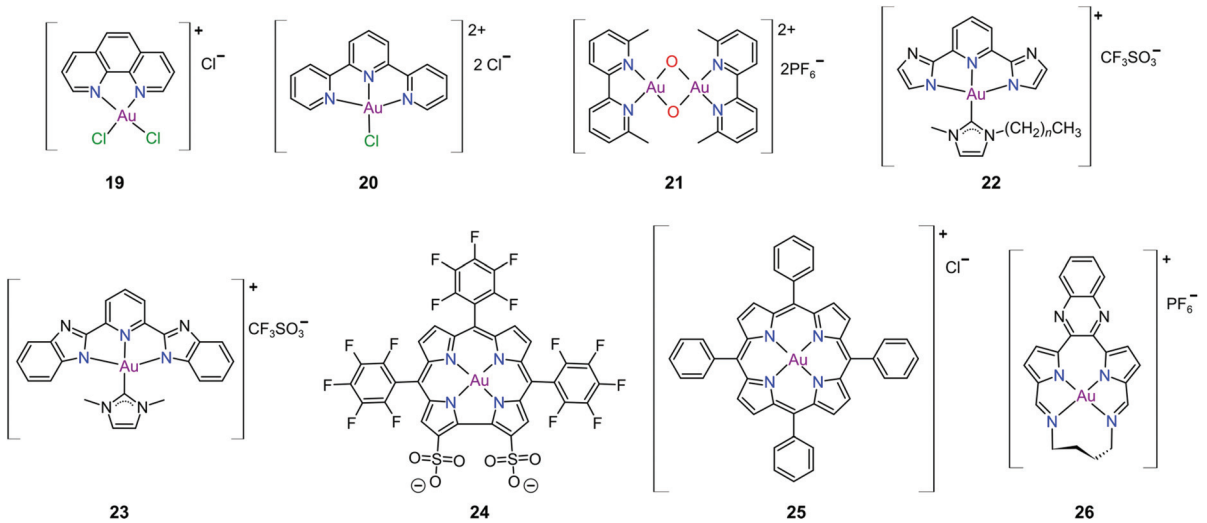


Fig. 12 Representative Au(III) chelates of polydentate N-donor ligands (**19–23**), a corrole derivative (**24**), a tetra(aryl) porphyrin derivative (**25**), and a bis(pyrrolide-imine) macrocycle (**26**). Ligands **24–26** effectively stabilize the Au(III) ion against reduction under physiological conditions.

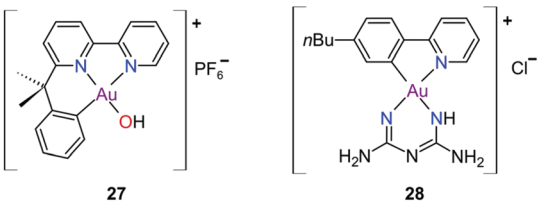
Table 3 Cytotoxicity data for Au(III) complexes **22–25** and **28–33** with various cancer- and healthy (nontumorigenic) cell lines. Where applicable, the calculated selectivity is indicated (*i.e.*, the ratio of the IC_{50} for healthy cells to the IC_{50} for cancer cells). For comparison, the IC_{50} values of cisplatin measured under the same conditions are shown italicized in brackets

Entry	Complex	IC_{50}[a] (μM): cancer cells (cell line, time[b])	IC_{50}[a] (μM): non-cancer cells (cell line, time[b])	Selectivity
1	**22**	1.4 ± 0.2 (HeLa, 72 h)[c], *(1.8 ± 0.1)*[f]		
2	**23**	14.4 ± 2.2 (HeLa, 72 h)[c], *(1.8 ± 0.1)*[f]		
3	**24**	19.7 (MDA-MB-231, 72 h), *(44.8)*		
4	**25**	0.3 ± 0.02 (HeLa, 48 h)[c], *(11.2 ± 1.0)*	0.91 (CCD-19Lu, 48 h)[c]	3.3
5	**28**	7.7 ± 0.1 (HeLa, 72 h)[c], *(5.2 ± 0.1)*	32.8 ± 2.6 (CCD-19Lu, 72 h)[c], *(36.2 ± 0.4)*	4.1
6	**29a**	3.4 ± 0.6 (HeLa, 72 h)[c], *(11 ± 1.0)*		
7	**29b**	7.2 ± 0.6 (HeLa, 72 h)[c]	18 ± 0.6 (CCD-19Lu, 72 h)[c]	2.5
8	**29c**	8.0 ± 0.5 (HeLa, 72 h)[c], *(11 ± 1.0)*		
9	**29d**	8.2 ± 0.5 (HeLa, 72 h)[c], *(11 ± 1.0)*		
10	**30**	0.04 ± 0.006 (HeLa, 72 h)[c], *(11 ± 1.0)*	1.6 ± 0.1 (CCD-19Lu, 72 h)[c]	37
11	**31a**	0.08 ± 0.007 (HeLa, 72 h)[c], *(11 ± 1.0)*	1.9 ± 0.1 (CCD-19Lu, 72 h)[c]	23
12	**31b**	0.2 ± 0.05 (NCI-H460, 72 h)[c], *(3.5 ± 1.0)*	25 ± 3.8 (CCD-19Lu, 72 h)[c]	147
13	**32**	0.3 ± 0.2 (HL60, 72 h)[d], *(3.7 ± 0.3)*	1.4 ± 0.4 (MRC-5, 72 h)[d], *(10.7 ± 3.0)*	4.5
14	**33**	2.3 ± 0.8 (MDA-MB-231, 48 h)[e]	8.6 ± 2.1 (EA.hy926, 48 h)	3.8

Cell viability data were reported as [a] the half maximal inhibitory concentration required to inhibit the growth of 50% of cells in the culture (IC_{50} values, μM) during [b] indicated time of drug exposure and were determined by either [c] the MTT assay (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), or [d] the MTS assay (MTS = 5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazolyl)-3-(4-sulfophenyl) tetrazolium, or [e] the crystal violet assay. [f] IC_{50} of auranofin measured under the same conditions.

The complexes reported by Buckley *et al.* in the 1990s (*e.g.*, **16**, Fig. 11) are stable against reducing agents such as ascorbic acid and GSH, whereas the similarly structured pyridine-based complexes (**17** and **18**, Fig. 11) were unstable in solution. These early organogold complexes, however, prompted widespread studies to develop multidentate ligand scaffolds for Au(III), which ultimately revealed that at least one C-deprotonated (C^-) ligand coordinated to the metal is sufficient to stabilize the Au(III) ion against reduction.[24,184,255] Representative examples of cytotoxic organogold(III) complexes chelated by tridentate (CNN)-(**27**) and bidentate (CN)-(**28**) ligands are shown in Fig. 13. Compared to cisplatin and several other (NN)-, (NNC)- and (NC)-Au(III) complexes in the series reported by Messori *et al.*,[273] **27** was among the most cytotoxic (entry 7, Table 2). Interaction with nucleic acids was found to be weak, but **27** does inhibit TrxR2 (0.28 μM) and interacts strongly with other proteins such as lysozyme and cytochrome *c*.[273] Proteomic studies were conducted to identify the effect of **27** on protein expression in whole cells and found that only a few proteins had modified expressions. Of those modified proteins, most of them were involved in the stress response of the cell and proteins associated with glucose metabolism were downregulated, identifying mitochondria as a key target in the cytotoxicity of this complex.[255] Compound **28** is a stable, water-soluble salt that is able to form Au^{III}–GSH adducts and induce ER stress. The *in vitro* cytotoxicity of **28** was evaluated against several cancer cell lines and although not as potent as the porphyrin complex **25**, this simple complex had marginally better selectivity than **25** (entries 4 and 5, Table 3).[274]

In pursuit of suitable Au(III) DNA-metallointercalators (to potentially mimic the mode of action of $[Pt(terpy)L]^+$), Che and co-workers synthesized structurally varied CNC Au(III) complexes based on 2,6-diphenylpyridine (**29–31**, Fig. 14). Conceptually, not only would the dianionic $[CNC]^{2-}$ ligand



27 28

Fig. 13 Representative examples of Au(III) chelated by tridentate CNN-donor (**27**) and bidentate CN-donor pyridine-based ligands (**28**).

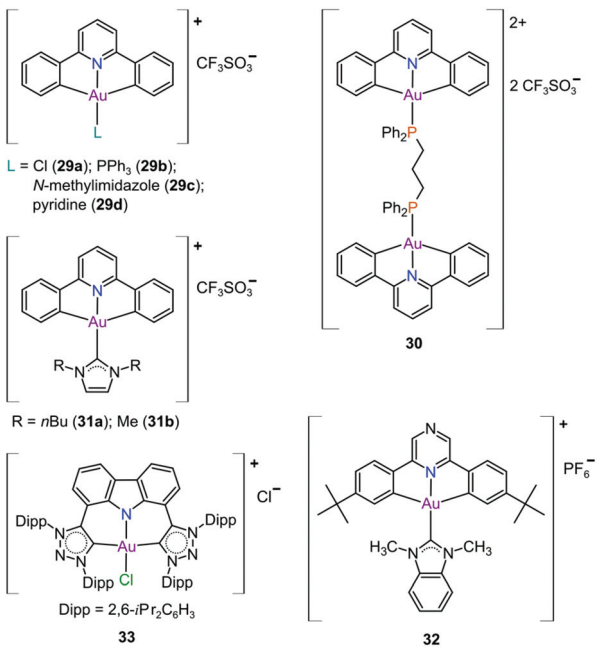


Fig. 14 Representative examples of cytotoxic Au(III) CNC pincer complexes.

scaffold provide sufficient stabilization for the Au(III) ion, but it also lends itself to diverse structural modifications. Although these modifications included varying the scaffold using 2,4,6-triphenylpyridine and 2,6-dinaphthylpyridine, structural adjustments of the ancillary ligand, L, in $[Au(CNC)L]^+$ ultimately had the largest impact on cytotoxicity (complexes **29a–29d** in Fig. 14 and entries 6–9, Table 3).[184,275,276] The mechanism of action was also influenced by structural variations of L. For instance, the *N*-methylimidazole (*N*-MeIm) complex, **29c**, intercalates double-stranded DNA and arrests the cell cycle in S-phase, the phase in which DNA replication takes place. Therefore, it has been suggested that **29c** induces apoptosis by inhibiting DNA replication. Conversely, the triphenylphosphine complex (**29b**) interacts only weakly with DNA.[184,275,276] The cytotoxicity of both **29b** and **29c** were drastically improved by modifying the mononuclear **29b** to a dinuclear Au(III) derivative (**30**, Fig. 14) by utilizing a bridging bis(diphenylphosphino)propane (μ-dppp) ligand[277] and substituting the *N*-MeIm ligand of **29c** with NHCs, resulting in complexes **31a** and **31b** (Fig. 14).[231] Complex **30** showed impressive cytotoxicity in the submicromolar range against several cancer cell lines (entry 10, Table 3) with a 37-fold selectivity ratio. Complex **30** is a potent TrxR inhibitor (2.7 nM, rat TxrR1) and induces ER stress.[277]

Complex **31a** is 100-fold more active against HeLa cancer cells (entry 11, Table 3) compared with **29c** (entry 8, Table 3). The dimethylated NHC Au(III) complex **31b** showed remarkable selectivity for non-small cell lung cancer cells (NCI-H460, entry 12, Table 3) relative to normal fibroblast cells (CCD-19Lu, entry 12, Table 3). Complex **31b** is able to intercalate into DNA and subsequently prevent topo I mediated relaxation of supercoiled DNA.[24,184,231] Moreover, multiple targets for Au(III) CNC NHC complexes of type **31** were identified by Che and coworkers using a clickable photoaffinity probe.[278] This study indicated that multiple proteins bind to type **31** cations, such as hsp60, vimentin, nucleoside disphosphatase kinase A, nucleophosmin, nuclease-sensitive element binding protein, and peroxiredoxin 1. Notably, all of these proteins have been identified as potential anticancer drug targets.[184,255,276]

The pyrazine-based CNC complex **32** also had high *in vitro* cytotoxicity with a 4.5-fold selectivity ratio (measured against healthy foetal lung fibroblast cells, MRC-5: entry 13, Table 3).[279] DNA binding studies revealed that **32** is capable of stabilizing G-quadruplex DNA secondary structures. Surprisingly, the caffeine analogue of **32** was 10 times less cytotoxic and this was attributed to lower cellular uptake.[279] The more polar caffeine-based ligand possibly hindered cellular uptake of the complex, which potentially occurs through passive diffusion.

The Au(III) ion of the sterically encumbered CNC pincer complex **33** is sufficiently stabilized by the bis-1,2,3-triazol-5-ylidenes and the anionic carbazolide ligand scaffold against reduction by GSH.[280] Furthermore, **33** showed promising selectivity during *in vitro* cytotoxicity studies (entry 14, Table 3). Further mechanistic studies revealed that **33** binds to dsDNA *via* partial intercalation, while *in silico* experiments suggested that **33** might play a role in the stabilization of DNA 3WJs due to its charge (+1) and shape complementarity.[280]

Since the discovery that Au(III) can be stabilized for use in biological media and protected against reduction under physiological conditions by judicious choice of appropriate ligand scaffolds, many more examples have been reported and mechanistically explored for their antitumour potential. In particular, porphyrin- and CNC pincer-stabilized Au(III) complexes show exceptional promise, although selectivity towards cancer over healthy cells remains a challenge calling for future solutions. Using non-toxic ligands such as NHCs generally improves selectivity. However, too few Au(III) complexes, including Au(III)-NHC derivatives, have been evaluated against healthy cell lines to currently draw any firm conclusions. NHCs, however, are strong σ-donors and have been shown to effectively stabilize both Au(I) and Au(III) under physiological conditions.[254–258] Indeed, the first crystal structure of an Au(I)-NHC protein adduct has recently been reported (Fig. 15).[281] The adduct involves the plant protein thaumatin (from *Thaumatococcus daniellii*), which was used as a generic globular protein model. In contrast to most other Au(I/III) complexes,

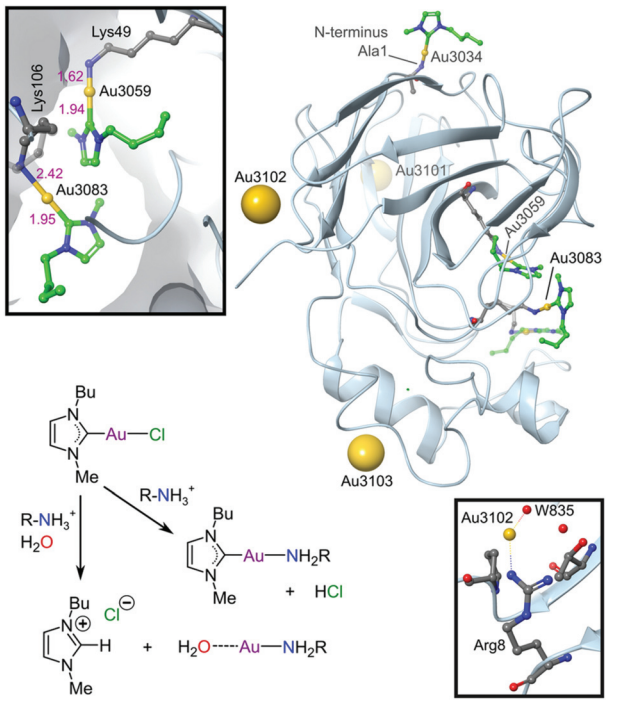


Fig. 15 X-ray structure (1.70 Å resolution) of the Au(I)-NHC adduct with thaumatin (PDB 5JVX). The three Au(I) ions bound to surface residues (Arg8, Lys97, Arg175) are indicated by large spheres. These Au(I) ions have water weakly coordinated trans to the protein N-donor ligand and the NHC has dissociated (presumably as the protonated imidazolium ion). Coordination of Au(I) to Arg8 and water 835 is indicated (lower inset) to illustrate a surface-bound NHC-free Au(I) ion. Three other amino groups (Ala1, Lys49, and Lys106) are coordinated to Au^I–NHC moieties (green carbon atoms). The upper inset displays the coordination environments for the Au^I–NHC moieties bound to Lys49 and Lys106. The reaction scheme summarizes the most likely coordination chemistry for the system.

where ligands are often lost upon binding to proteins,[282] the NHC moiety in this case was retained by half of the bound Au(I) ions. Since thaumatin lacks His and free Cys residues (Cys in thaumatin is involved exclusively in S–S bridges), the Au(I) complex interacts with several solvent-exposed lysine and arginine residues. We have depicted the likely coordination[283] and acid–base chemistry for NHCs[284] leading to the two different types of protein-bound Au(I) ions in this system (Fig. 15), rectifying this omission from the original literature.

Outlook

The ability of cytotoxic gold-based anticancer agents to elicit apoptosis has been amply illustrated by both Au(I) and Au(III) complexes over several decades. Judicious choice of the ligand scaffold for the metal ion is critical to achieving this goal and many suitable ligand classes now exist, including conventional N-heterocyclic and triazolylidene-based mesoionic carbenes. The attractive features of carbene ligands, namely their ease of preparation, good stability under physiological conditions, structural diversity, and functional group tunability, foreshadow further exploitation of this class of C-donor ligands in the design of ever more specific and better-targeted metallodrugs.

Given the need to develop anticancer drugs with fewer side-effects,[110] the use of specific macromolecule-, organelle- or cancer cell-targeting groups (*e.g.*, glucose, folic acid, biotin, oestrogen, peptides, proteins, polymers, micelles, nanoparticles, *etc.*),[285–287] perhaps as part of an ancillary ligand such as a thiolate coordinated *trans* to a compact, exchange-inert carbene in a two-coordinate Au(I) complex,[288] might produce metallodrug candidates better suited to preclinical development. The idea of adding targeting groups or other functional moieties, such as fluorophores, to Au(I)–carbene derivatives has indeed been recently explored. Specifically, the cytotoxic acridine derivatives **34–37** (Fig. 16) were found to localize and fluoresce predominantly in tumour cell lysosomes.[289] Some localization within the nucleoli was also observed, consistent with nuclear uptake of the complexes. Complexes **36** and **37** had good IC_{50} values against MiaPaca2 cells (human pancreatic cancer) of 2.8 ± 0.8 and 3.4 ± 0.8 μM, respectively, and did not disrupt mitochondria,[289] as found with several other NHC–Au(I) complexes.[222,290] The advantage of fluorescently labelled compounds is that their biodistribution can be imaged at high resolution by confocal microscopy and important mechanistic information regarding their cellular fate straightforwardly delineated.

For metallodrug design more broadly, the challenge going forward will be to use *in silico* design methods and conventional discovery techniques with suitable metal complexes to target specific proteins, for instance TrxR2 in mitochondria[188]—exclusively in cancer cells. To do this will require selectively targeting: [1] tumour cells (thereby reducing general toxicity), [2] a specific organelle within the cytoplasm (the mitochondria), and [3] the active site Cys-Sec residue pair[291] of TrxR2. In short, a molecular recognition trifactor on top of

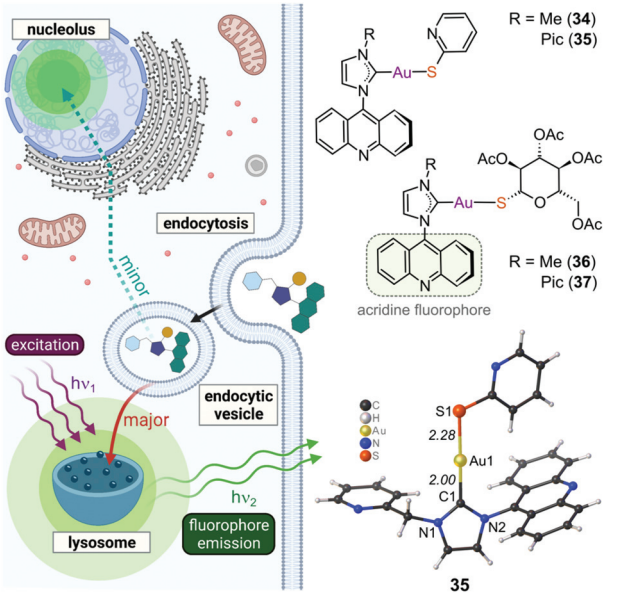


Fig. 16 Illustration of the structures of selected cytotoxic NHC–Au(I) thiolate complexes with an acridine fluorophore tag that localize mainly in tumour cell lysosomes and, to a lesser extent, in the nucleolus. The complexes are readily detected by confocal microscopy (405 nm excitation). Localization within lysosomes indicates a probable endocytosis internalization pathway for the compounds. The X-ray structure of **35** is shown at the lower right (CSD code AVAJIS, ref. 289). The bulky substituted NHC ligand probably prevents **34–37** from targeting thioredoxin reductase.

achieving optimal ADMET (absorption, distribution, metabolism, excretion, and toxicity) parameters *in vivo*.[292] The task is clearly formidable, but careful analysis of existing strategies alongside innovative new ideas, coupled with artificial intelligence algorithms[293,294] to process large data sets, might yield some truly successful Au(I/III) metallodrug candidates in the next two decades.

Author contributions

DvdW prepared the initial manuscript draft and figures. OQM created the final versions of all figures, some *de novo*, and others by reconceptualizing and artistically extending notions present in DvdW's draft figures. All three authors co-wrote the final draft of the paper.

Conflicts of interest

There are no conflicts to declare.

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