A focus on the biological targets for coinage metal-NHCs as potential anticancer complexes

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Abstract Metal complexes of N-heterocyclic carbene (NHC) ligands are the object of increasing attention for therapeutic purposes. Among the different metal centres, interest on Au-based compounds started with the application as anti-arthritis drugs. On the other hand, Ag(I) antimicrobial properties have been known for a long time. For Au(I)/Au(III)-NHC and Ag(I)-NHC anti-tumour and anti-proliferative properties have been quite recently demonstrated. In addition to these and as for Group 11, copper is a much less investigated metal centre, but a few papers underline its pharmacological potential. This review wants to focus on the different biological targets for these metal-based compounds. It is divided into chapters which are respectively devoted on: i) mitochondria and thiol oxidoreductase systems; ii) other relevant enzymes; iii) nucleic acids. Examples of representative coinage NHCs for each of the targets are provided together with significant references on recent advances on the topic. Moreover, a final comment summarises the aspects enlightened by each chapter and provides some hints to better understand the metal-NHCs mechanistic behaviour based on structure-activity relationships.

Keywords: N-heterocyclic carbene; metal-based complexes; gold; silver; mode-of-action; thioredoxin reductase

1. Introduction

A great number of possible biological targets (in particular subcellular structures in the eukaryotic cell) have been identified as relevant for anticancer therapies [1]. Among these, some have been successfully reached by prospective metal-based anticancer compounds, as widely documented

for the cornerstone anticancer metal complex, the diamminedichloridoplatinum(II) (cisplatin or CDDP) [2,3]. On this basis, many early studies were focused on the optimisation of alternative cisplatin compounds [4], and this is a research line still active nowadays [5,6]. For these systems, able to damage polynucleotides by creating adducts or crosslinks, natural double-stranded DNA is regarded as the main target (Figure 1). Moreover, peculiar nucleic acid geometries such as G-quadruplex structures are since some years put under the spotlight [7,8].

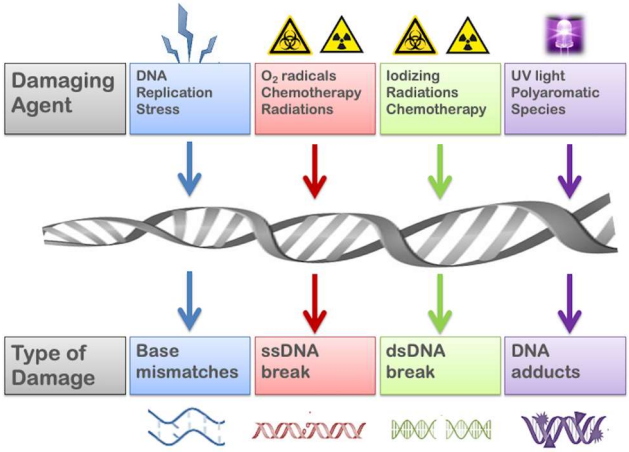

Figure 1. DNA damaging agents and types.

Besides genomic targets, inhibition of cancer-sustaining proteins is an important anticancer weapon and metal complexes offer unique features for designing effective protein inhibitors with anticancer potentialities [9,10]. Also, mitochondria are crucial cellular organelles not only for the energy supply of the cell but also for signalling and apoptosis regulation (Figure 2). In fact, impairment of the activity of cancer cell mitochondria, whose metabolism is usually altered, could pave the way for overcoming resistance and developing more selective drugs [11,12].

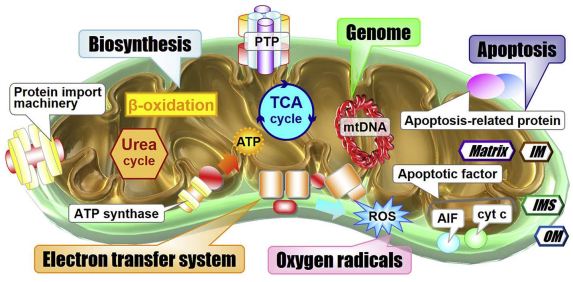

Figure 2. The various functions of mitochondria. Reprinted with permission from ref [11].

Within this frame, complexes with N-heterocyclic carbene ligands (NHCs, Figure 3) have found application as potential anticancer compounds [13]. They are cyclic carbenes characterized by a two-coordinate sp^2 hybridized carbon atom bearing a lone pair which is stabilised by two π-donating heteroatoms (at least one nitrogen) [14]. Through functionalization of the ring, targeting properties can be fine-tuned [15,16], together with steric and electronic features [17]. Another advantage is that NHCs can coordinate practically all transition metals [18,19].

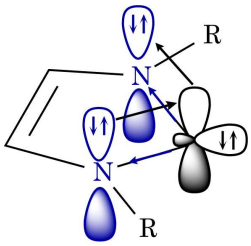

Figure 3. Chemical structure of a generic N-heterocyclic carbene ligand.

Gold-based medicine has a long history tracing back to ancient times [20,21] but, more importantly, gold compounds have been mostly used recently for the cure of rheumatoid arthritis [22,23]. Despite the relevance of anti-arthritis chrysotherapy is decreasing, its discovery was a turning point for the repurposing of gold drugs in the treatment of cancer and infectious diseases (human immunodeficiency virus, amoebiasis) [24–26]. Silver salts were mainly employed for their antimicrobial effects [27]. Nowadays, silver preparations are found in wound dressings and creams to prevent infections in burns or wounds [28,29], and the interest in silver antimicrobials is now experiencing a new impulse for overcoming the issue of bacterial resistance [30]. More recently, it has been pointed out that silver also exerts antiproliferative efficacy on cancer cells. In this regard, silver organometallics, which could ensure an increased solubility and stability with a slower release of the silver ions, are considered very interesting [31,32]. Given the promising results coming from gold and silver metal compounds, the attention moved the other component of Group 11, copper. Different researchers highlighted that copper may be an interesting substitute for platinum, as this endogenous metal could in principle be better tolerated [33] and natural biological pathways detoxify when necessary [34]. Unfortunately, investigations of the medicinal activity of copper NHCs are nowadays still scarce [35]. On the other hand, there is evidence that the flexible Cu(I)/Cu(II) redox behaviour may tune metal complexes activity/toxicity [36] and the altered copper metabolism between normal and tumour cells may be a basis for increased selectivity [37].

This review will focus on the main biological targets which have been individuated as relevant for the anticancer activity of Group 11 metal N-heterocyclic carbenes. Indeed, coinage metal compounds are among the most promising alternatives to platinum-based treatments [38].

2. Mitochondria and thiol oxidoreductase systems as targets

Mitochondria are regarded as cells' powerhouse accounting for about 80% of adenosine triphosphate (ATP) production in normal cells [39]. Recently, their role in the triggering of the intrinsic apoptotic pathway has been elucidated [40]. The 'non-return' point of this pathway is the mitochondrial outer membrane permeabilization (MOMP) leading to the release in the cytosol of cytochrome c and other pro-apoptotic proteins located in the transmembrane space. Two pathways occurring in response to various pro-apoptotic signals were individuated as responsible for MOMP. One involves the direct interaction of proteins belonging to the Bcl-2 family with the outer mitochondrial membrane [41]. The other one leads to the so-called membrane permeabilization transition (MPT) via the opening of a pore in the inner membrane [42]. As a consequence, the transmembrane potential is depleted and mitochondria inner membrane swelling occurs, leading to the break of the outer membrane and consequent MOMP. However, some cancer cells are intrinsically resistant to MOMP and thus unresponsive to pro-apoptotic stimuli that lead to this specific cell death pathway [43,44]. Thus, in the light of the above-described mechanisms, novel ways can be explored to develop drugs which directly affect the mitochondria and triggering apoptosis by evading these upstream regulatory mechanisms which often involve overexpression of Bcl-2 anti-apoptotic proteins or p53 deregulation [45,46]. p53 is a very important transcription factor activated following DNA damage or other stress stimuli; it is implicated in numerous functions including maintenance of genomic stability, cell-cycle inhibition, tumour suppression and regulation of apoptosis [47,48]. Some differences between normal and tumour cells could be exploited for selective targeting [49]. For instance, it is now known that the mitochondria of cancer cells have elevated transmembrane potential ($\Delta\Psi_m$) [49,50]. The class of anticancer agents known as delocalized lipophilic cations (DLCs) are able to accumulate in mitochondria of cancer cells owing to their lipophilicity and charge [50]. DLCs based on silver and gold NHCs have been prepared and tested and some will be presented in this paragraph (see Figure 4) [51]. An alternative strategy for the delivery of metal-based compounds to mitochondria involves bioconjugation with peptides recognized by mitochondria protein import machinery or peptides and peptoids able to accumulate inside these organelles [52,53]. Accumulation of DLCs in mitochondria can cause toxicity by affecting different pathways [54–56]. Among these, inhibition

of the enzyme thioredoxin reductase has gained a central role in prospective anticancer strategies [46,57].

Mammalian thioredoxin reductase (TrxR) is a high molecular weight (50 kDa each subunit) homodimeric flavoprotein which belongs to a thiol oxidoreductase system deputed to the reduction of hydrogen peroxide (Figure 4). Each subunit contains a redox-active site accommodating a flavin adenine dinucleotide (FAD) molecule and a catalytic site with one selenocysteine (Sec) and one cysteine (Cys) amino acid residue [58]. These residues can undergo reversible oxidation through the formation of Se-S bond, shuttling electrons to the principal substrate of the enzyme, i.e. thioredoxins (Trx). These latter are a family of redox-active small peptides (10-12 kDa) which are involved in the reactive oxygen species (ROS) balance regulation and the viability and cell proliferation signalling [57]. The Trx system isoforms are present in cytoplasm and mitochondria. It has been shown that some types of cancers overexpress TrxR [59] and that inhibition of this selenoenzyme can trigger apoptosis [60,61]. The more acidic and softer Se^- is considered more prone to electrophilic attack by soft metal Lewis acids such as gold. Generally, gold(III) compounds show a lower inhibitory potency than gold(I) complexes but still appreciable. In a pivotal paper by Bindoli and co-workers, it was reasonably proposed that Au(III) compounds may act with a different mechanism on the TrxR that not involves the direct metal binding on Sec and/or Cys amino acid residues, but a probable redox pathway causing oxidative damage of the enzyme's active site [62,63].

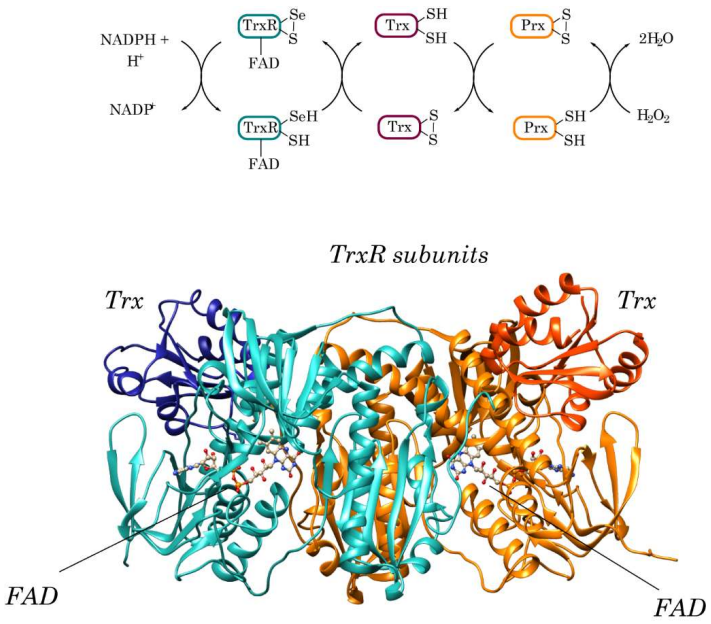

Figure 4. Thioredoxin system and crystal structure of the complex between human TrxR and Trx (PDB 3QFB), see also reference [58].

2.1 Mitochondria directed NHCs: delocalized lipophilic cations (DLCs) and neutral monocarbenes

An early interesting finding about NHCs antimitochondrial activity was reported by Barnard and others [64,65]. The accumulation of dinuclear gold carbene complexes of imidazolylidene-linked cyclophanes in the organelles was measured by inductively coupled plasma-mass spectrometry (ICP-MS) and Ca^{2+} sensitive mitochondrial membrane permeabilization (MMP) was observed. Compound **1** (Figure 5) was the most effective in the series. The same group prepared a series of cationic gold(I) NHC complexes of the type $[(R_2Im)_2Au]^+$ (compounds **2**-**6**) which had lipophilicity ranging from -1.09 to 1.73 and behaved as DLC. Indeed, it was found that there is a direct correlation between $LogP_{ow}$ (the partition coefficient between octanol and water), the accumulation in the mitochondria of cancer cells and the antimitochondrial activity (measured as the rate of MMP) [66]. Therefore, further development consisted of the tuning of the lipophilicity around the intermediate value. From this latter work, compound **3** ($LogP_{ow}$ = -0.83) [66] emerged as the optimum in terms of selectivity towards breast cancer cells if compared to compounds **7** and **8** [67]. Moreover, nuclear magnetic resonance (NMR) experiments in the presence of Sec and Cys pointed out that these compounds could undergo a two-step ligand exchange process. The higher rates were measured for less bulky substituents in the presence of Sec. This finding explained the high selectivity in the inhibition of the selenoenzyme thioredoxin reductase TrxR if compared to the one of glutathione reductase (GR), which contains a Cys in the active site. TrxR inhibition measured on cell lysates justified antimitochondrial effects and ensuing cytotoxicity [67]. Comparison of the biological activity of the cationic compound **3** with the related neutral monocarbene **9** confirmed that an increased membrane potential in cancer cells drove mitochondrial accumulation and cancer cell selectivity for this charged lipophilic complex. The same did not happen for the neutral complex, which showed similar cytotoxicity towards both tumorigenic and non-tumorigenic cell lines [49]. Cisnetti, Gautier and co-workers devised an 'autoclick' strategy for the functionalization of copper NHCs; after transmetalation, the neutral fluorescent gold NHC **10** was obtained and demonstrated to localize in mitochondria [68]. More recently, Arambula and co-workers synthesized heteroleptic gold NHCs bearing carbamate functional groups achieving a rich molecular diversity [69]. The fluorescence of the two carbenes **11** and **12** allowed confirmation of the mitochondrial localization. Of particular interest is the doxorubicin-functionalized NHC **12**: coordination to gold(I) redirects doxorubicin from the nucleus to mitochondria. Indeed, it has been proposed that this could be interesting for limiting the cardiotoxic effect of this chemotherapeutic agent [70].

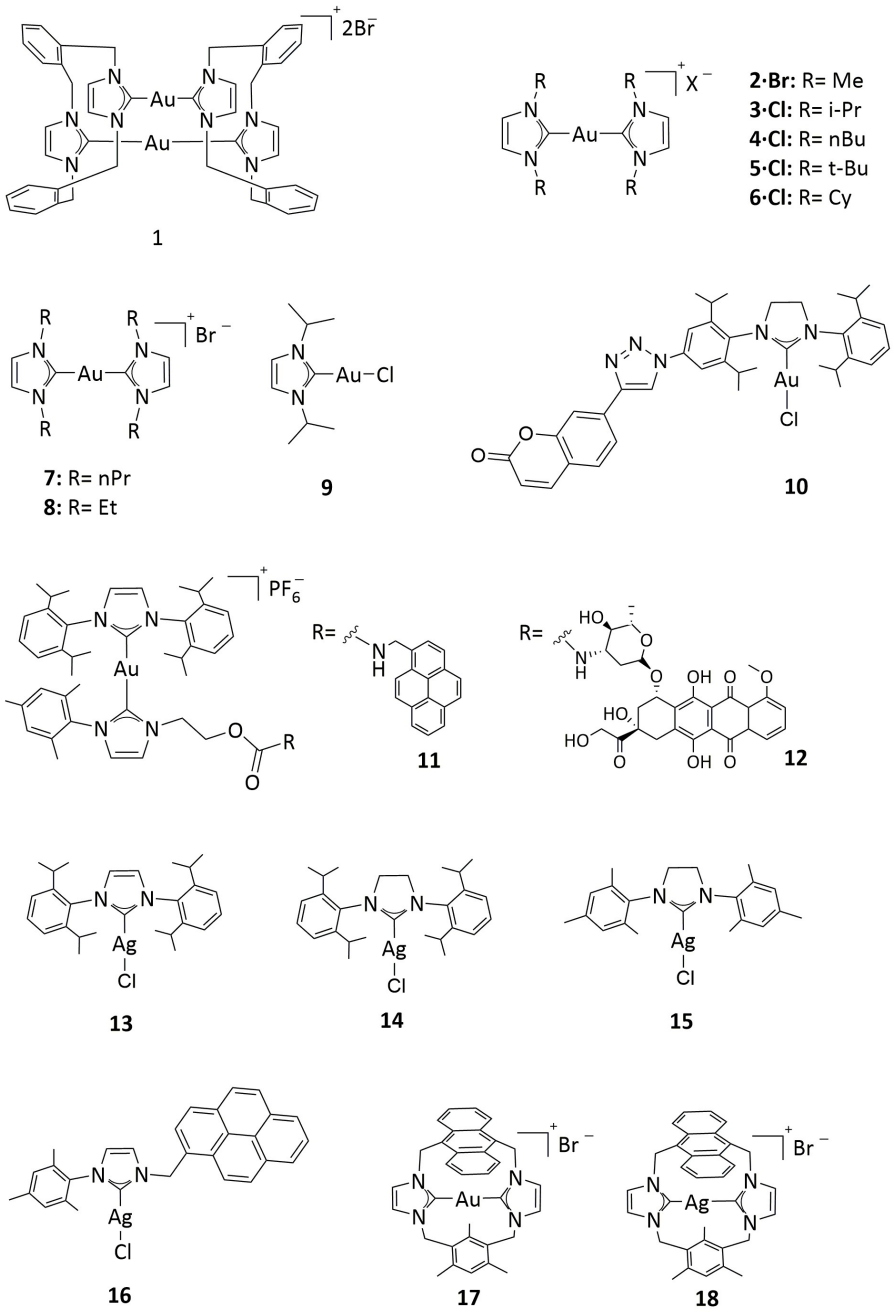

Figure 5. Chemical structures of metal NHCs targeting mitochondria.

As for silver, Eloy et al. reported a panel of fourteen Ag(I)-NHCs [71]. **13** and **14**, endowed of bulky aromatic wingtip substituents, elicited the most promising antiproliferative activity against various cancer cell lines followed by compound **15**. Subsequent experiments showed that these complexes do not cause reactive ROS overproduction but trigger mitochondrial $\Delta\psi_m$ loss in human leukaemia HL60 cells with related mitochondrial pores opening and release of pro-apoptotic factors. Indeed, results showed that apoptosis was due to the caspase-independent mitochondrial apoptosis-inducing factor (AIF) pathway activation.

The synthesis of the fluorescent analogue **16** was employed to demonstrate a mitochondrial localization of the complexes. This, and the among mentioned work [68], highlight how also carbenes with a lack of net charge can target mitochondria. These complexes are more prone to ligand exchange reactions and, as discussed in the next paragraph, some have been individuated as potent inhibitors of both the cytosolic and mitochondrial forms of TrxR.

An interesting report by Lin and others highlighted differences in the mechanism of action of silver(I) and gold(I) carbenes with the same NHC ligand derived from fluorescent cyclophanes [72]. **17** and **18** (the gold(I) compound to higher extents) accumulate in mitochondria, cause depolarization of the transmembrane potential and ensuing apoptosis. However, the apoptotic pathway due to the gold(I) carbene is ROS and caspase 3/7 dependent while the programmed cell death due to the silver(I) carbene is not. Caspases 3/7 are proteolytic enzymes that are activated when some apoptotic death pathways are triggered. Also, the gold(I) compound is more cytotoxic and selective.

2.2 NHC complexes inhibiting thioredoxin reductase (TrxR)

2.2.1 Gold(I) NHCs Several gold and silver carbenes have been individuated as potent inhibitors of purified and cellular TrxR so far [60,73,74]. An interesting work reported by Rubbiani and others in 2011, compared the *in vitro* biological activity of NHC-Au(I)-L type compounds **19**-**21** (Figure 6), with NHC = 1,3-benzimidazole-2-ylidene and L = NHC, PPh_3, Cl, showing how the bovine serum albumin protein (BSA) binding and TrxR inhibition were related to the Au-L bond dissociation energy [75]. The Au-Cl bond was the most unstable and, as expected, the correspondent complex was the most potent TrxR inhibitor. Conversely, the charged and lipophilic complexes with L = NHC, PPh_3 had an increased mitochondrial and cellular accumulation which explained their more potent cytotoxicity if compared to the neutral complex. These complexes displayed strong antimitochondrial effects that could possibly be related to additional mechanisms not involving mitochondrial TrxR inhibition. Moreover, it is worth to mention that these carbenes also displayed selective inhibition of TrxR compared to glutathione reductase (GR) and glutathione peroxidase (GPX), another selenoenzyme. Some selectivity towards cancer cell was only observed for the biscarbenic compound. Additionally, in collaboration with the group of Messori, the reaction of these **19**-**21** with the C-terminal peptide of human TrxR was investigated with electrospray ionization mass (ESI-MS) spectrometry [76]. This provides structural evidence for the binding of the gold carbenes to the selenocysteine and preference over binding to cysteine. Such structural evidence confirmed what found for other gold complexes and partially remedies the lack of a crystal structure of TrxR with an inhibitor [77]. Notably, the chloride **21** shows the

most extensive metalation, followed by the dicarbene. This order does not reflect nor the binding energy of the Au-L bond nor the inhibition potency previously found [75]. In a later work, Ott and co-workers analysed in detail the signalling pathways and metabolic features of cancer cells treated with the model compound **22** [78].

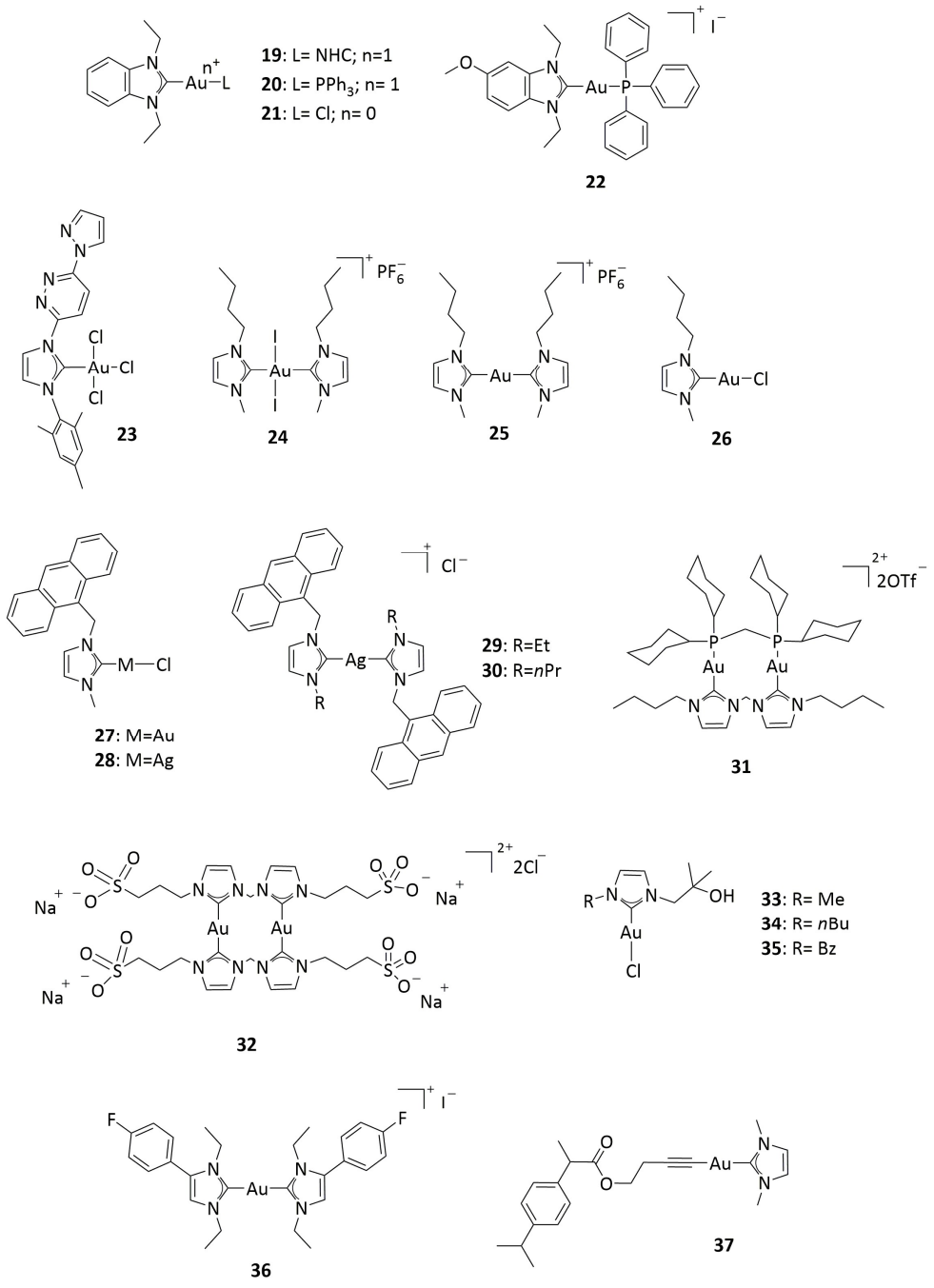

Figure 6. Chemical structures of gold(I) NHC TrxR inhibitors

Results evidenced three different effects contributing to the triggering of apoptosis: (i) direct depletion of mitochondrial respiration; (ii) TrxR inhibition and (iii) indirect DNA damage (mainly

due to oxidative stress arising). In 2013 the same group reported an extensive structure-activity relationship analysis on gold(I)/gold(III) NHC inhibition of TrxR and correlated it with cytotoxicity [79]. In all cases, gold(I) complexes were more effective enzyme inhibitors, but the gold(III) chloride **23** was the most cytotoxic of the series. Most of the compounds displayed low IC_{50} values for both TrxR inhibition and cytotoxicity, but an unambiguous correlation lacked. Different uptake, metabolization or activation pathways could account for these results, depending on the characteristics of the complexes. Overall, a scarce dependence of the biological activity on the oxidation state is outlined for these type of compounds [79–84]. These findings may suggest a limited stability of these complexes *in vitro*. *In vivo* reduction of the gold(III) NHC **24** to its gold(I) NHC precursor **25** was recently demonstrated by positron emission tomography (PET) and inductively coupled plasma mass spectrometry (ICP-MS) biodistribution experiments on a radiolabelled analogue [85]. Additionally, *in vitro* experiments showed that reduction to the corresponding gold(I) NHC is important for exerting a full antiproliferative activity. The dicarbene complex **25** maintains its structure also in the presence of biologically relevant reducing agents such as glutathione (GSH) [86] and showed potent cytotoxic properties *in vitro* against several cancer cell lines [86,87]. When incubated with model proteins, e.g. cytochrome c and lysozyme, bearing solvent-exposed sulfurs or other coordinating groups, no interaction is observed. Conversely, after the release of the NHC ligands, a naked gold(I) atom was found covalently bound to the protein target, in this case the copper trafficking protein Atox-1 [86]. A comparative proteomic investigation of the antiproliferative activity of **25** and **26** against A2780 human ovarian cancer cells pointed out the increased antiproliferative activity of the dicarbene with respect to the monocarbene analogue. The report evidenced that compound **25** behaved as a DLC inhibiting TrxR and thus causing mitochondrial metabolism impairment [88]. Casini, Rigobello and coworkers [89], and more recently Messori and colleagues [90] have shown that anthracene functionalized gold(I) (and silver(I)) carbenes have potent antiproliferative properties, they are potent TrxR inhibitors and, taking advantage from to the fluorescence of the anthracenyl label, can be visualized inside cells. Interestingly, compounds **27** and **28** reported by Casini and Rigobello showed an increased accumulation in the nucleus while the silver biscarbene **29** reported by the group of Messori is shown to localize principally in vesicles within the cytoplasm. Notably, the silver carbene **28** is a more potent TrxR inhibitor if compared to the gold analogue **27** and selectively cytotoxic towards cancer cells. The ESI-MS of the adduct of a synthetic C-terminal dodecapeptide of hTrxR(488-499) with the two related anthracenyl functionalized silver NHCs **29** and **30** evidenced that the NHC ligand alone with no metal atom was bound [90]. At variance, no adduct was found in the case of the monocarbene **28**. The dinuclear compound **31**

exhibited promising properties [91]. It shows limited reactivity towards bovine serum albumin protein (BSA) which has positive effects on the cytotoxicity if compared to the other complexes reported. At the same time, it is a tight binder of TrxR active site: ESI-MS and NMR experiments demonstrate that the two gold centres bind the Sec and Cys groups with release of the bis NHC ligand. Moreover, it inhibited the growth of Hela xenografts and mouse melanoma, without evident toxicity. Rarely observed anti-angiogenesis effects *in vivo* are also pointed out. For the same complex, inhibition of the sphere-forming ability of cancer stem cells was observed, indicating a tendency to contrast metastasis formation [91].

In 2017 a series of hydrophilic gold(I) carbenes were characterized as TrxR inhibitors [92]. The dicarbene **32** is poorly reactive toward nucleophiles and, accordingly, it lacked inhibitory activity against TrxR and cancer cell proliferation. On the other hands, hydroxylated monocarbenes **33-35** were cytotoxic and strong selective inhibitors of TrxR if compared to GR considering the purified enzyme and ovarian cancer cell extracts. The affinity of the compounds for Sec and Cys was demonstrated by means of biotinylated iodoacetamide (BIAM) switch assay and competition mass spectrometry (MS) experiments in the presence of other amino acids. MS speciation experiments of **33** highlighted the propensity to undergo ligand exchange reactions: the formation of the corresponding dicarbene was observed in water, while coordination of NH_3 was observed in ammonium carbonate buffer. In all the experiments the $Au(NHC)^+$ moiety remained intact. A comparative work between various Au(I) complexes evidenced the improved antiproliferative properties of the dicarbene **36** [93]. Interestingly, it was demonstrated how its poor reactivity towards serum proteins positively affected the uptake and cytotoxicity. As expected, the carbene **36** is the least reactive towards purified rat liver mitochondrial TrxR, but still a good inhibitor. This compound is inactive towards bacterial TrxR devoid of the Sec residue which probably facilitates ligand exchange reactions in the case of mammalian TrxR. Probable binding poses of the compounds in the active site of mammalian TrxR are obtained with molecular docking studies. Very recently two gold(I) complexes coordinated to an alkynyl ibuprofen ligand and an NHC (see compound **37**) or PPh_3 ligand were reported [94]. Both compounds are nanomolar inhibitor of purified cytosolic and mitochondrial TrxR while being at least 240-fold less active towards GR. Both complexes are more selective towards cancer cells than cisplatin or auranofin but the phosphinic complex displays more potent antiproliferative activity. Experiments correlated this behaviour with an increased cellular uptake, and density-functional theory (DFT) calculations suggest a dependence on the ligand exchange favourable thermodynamics.

2.2.2 Gold(III) and gold-containing heterometallic NHCs The versatility of metal NHCs also allowed the preparation of heterometallic compounds (Figure 7). Compound **38** shows

improved TrxR inhibition and increased cytotoxicity towards Caki-1 human clear cell renal carcinoma line and HCT116 human colon cancer cell line if compared to monometallic precursors demonstrating the synergistic effect of the two metals [95]. With the aim of designing complexes that could impair the antioxidant system via different pathways, Arumugan and co-workers prepared ferrocene linked Au(I)NHC complexes [96]. A synergistic effect concerning lung cancer cell proliferation inhibition was observed. Also, antiproliferative properties and ROS elevation increased with the ferrocene content. Complexes **39** and **40** were also confirmed to inhibit TrxR in live cells and RNA microarray gene expression pointed out that ROS increase after treatment with **40** triggers endoplasmic reticulum stress response pathways.

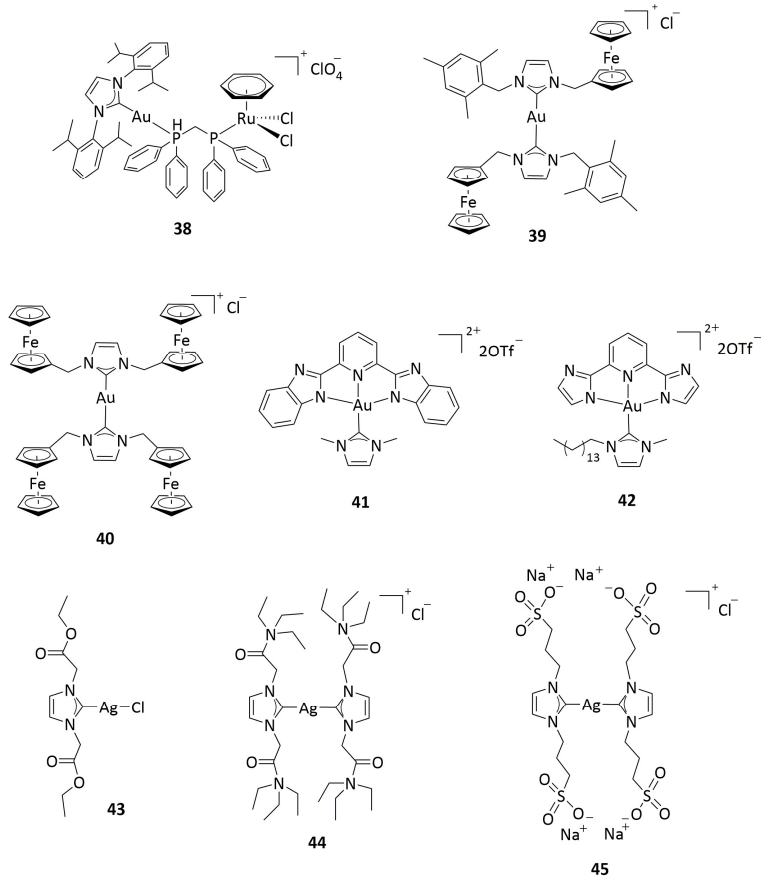

Figure 7. Chemical structures of heterometallic and silver(I) NHC TrxR inhibitors

As for gold(III), Che and coworkers designed a series of gold(III) NHC prodrugs exploiting their affinity for intracellular thiols [97]: a tridentate N^N^N ligand conferred relative stability towards ascorbic acid or BSA, but in the presence of low molecular weight thiols such as GSH, reduction with the release of HN^N^NH ligand and Au(I)(NHC)(GS) occurred very rapidly (see compounds **41** and **42**). In the case of **41** the HN^N^NH free ligand was emissive in the visible region when

not coordinated to gold. Thus, fluorescent microscopy was employed to prove intracellular thiol dependent reduction and localization in mitochondria (Figure 8). TrxR inhibition was measured on cell lysates and correlates well with the observed cytotoxicity. *In vivo* experiments on mice bearing HeLa xenografts showed tumour growth inhibition after treatment with the most active compound **42**, characterised by the presence of a 15-residue alkyl chain on the NHC.

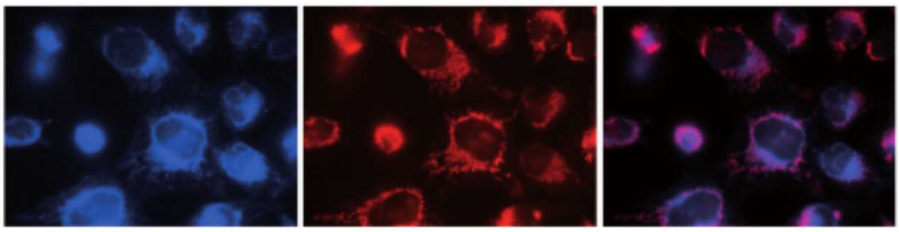

Figure 8. Fluorescence images of the intracellular localization of **41** (20 μM) in HeLa cells (left, 365 nm excitation), mitochondria-specific Mito-tracker Red stain (middle, 546 nm excitation), and the merged image (right). Adapted from ref [97].

2.2.3 Silver NHCs The work concerning the silver carbene **28** [89], already mentioned, and other silver NHCs [98] show that silver carbenes can have a more favourable biological activity than their gold analogues. Despite this is not always the case [99], there is much room for research as silver complexes are historically studied as antimicrobials, while their antitumour properties are only very recently being underlined. However, most of the reports are limited to the *in vitro* antiproliferative properties and mechanistic aspect are not fully elucidated [100,101]. The antiproliferative properties of water-soluble silver NHCs were investigated by Santini and colleagues [98,99]. The ester- amide-functionalized silver carbenes **43** and **44** were found to be more cytotoxic than the gold analogues. Their activity was accompanied by very potent inhibition of TrxR being compound **44** the most effective; a copper analogue was prepared but its instability hampered the investigations on its biological activity [98]. Sulfonated dicarbene **45**, was more cytotoxic than cisplatin and activity was maintained against cisplatin-resistant cells. Again, potent TrxR inhibition was observed also in cell lysates. Further mechanistic studies confirmed that the observed apoptosis could be related to the inhibition of this key enzyme [99].

3. Other enzymes as targets

Despite in most cases mitochondrial damage and TrxR inhibition could account for the antiproliferative activity of gold(I) NHCs, also other putative targets have been identified. For example, the imidazole-2-ylidene gold chlorides **46**-**48** (Figure 9) have been demonstrated to

inhibit protein tyrosine phosphatases (PTPs) in cell-free assays, in leukaemia cells, and in primary mice thymocytes [102].

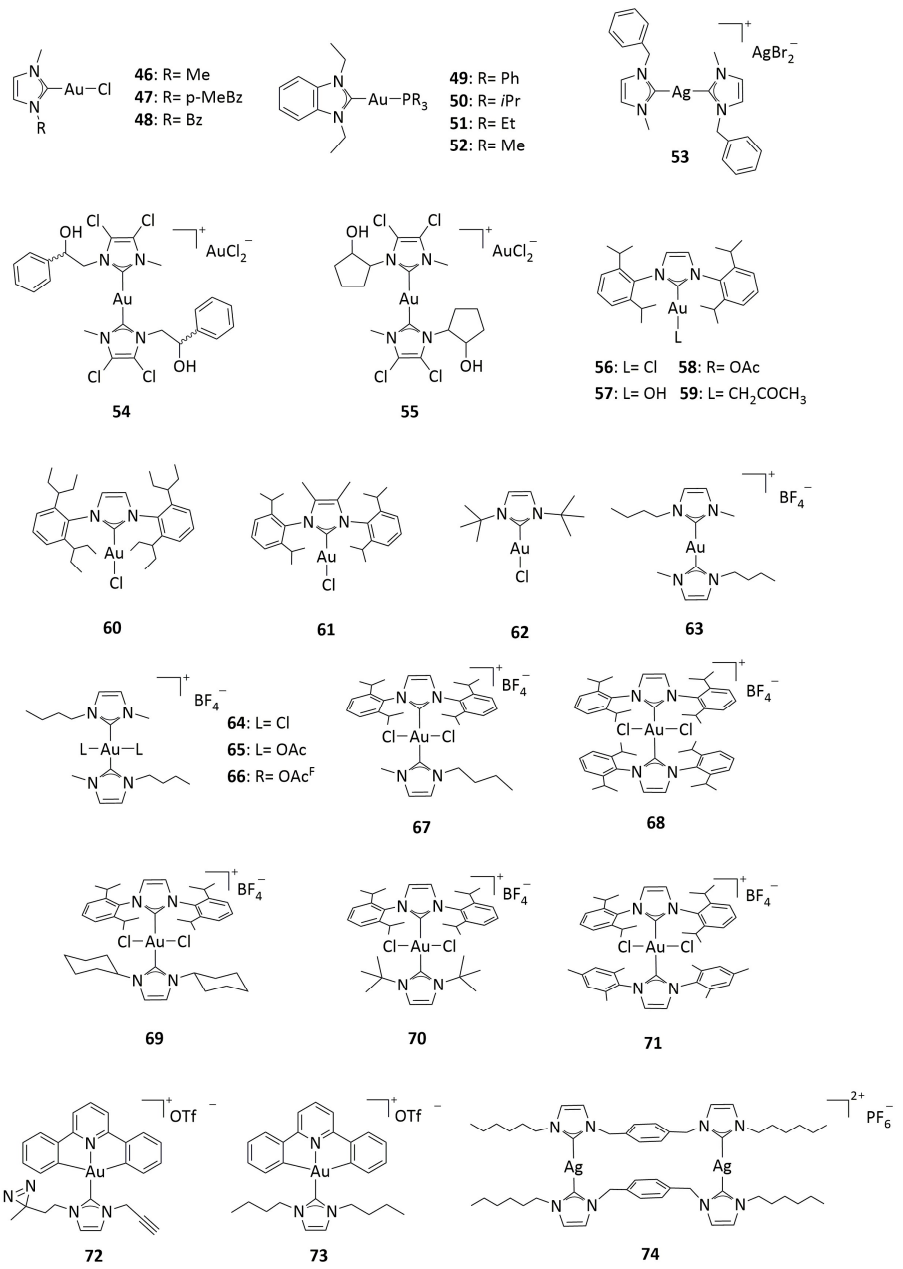

Figure 9. Chemical structures of metal NHCs directed to other enzymes.

PTPs are a family of enzymes featured by a cysteine residue in the active site. They catalyse the removal of phosphate groups from tyrosine residues in proteins and have key regulatory roles in signalling pathways and in cell-cycle control. Their importance in cell growth, proliferation and differentiation makes them interesting targets for diseases such as cancer or autoimmune disorders. Experiments suggested a competitive, reversible inhibition. In all cases, the NHCs were more potent inhibitors than auranofin. Moreover, **47** and **48** were the most potent inhibitors,

suggesting the importance of the benzyl side chain. Cellular studies showed that these compounds have good activity against PTPs as PTP-PEST and LYP but poor affinity for PTP - CD45 antigen, although inhibition of other PTPs could not be ruled out. The series of gold(I) carbenes with phosphane ligands **49**-**52** reported by Ott, Casini and coworkers exhibited nanomolar inhibitory activity against the zinc finger protein poly[ADP-ribose] polymerase-1 (PARP-1) and TrxR [103]. PARP-1 is an enzyme implicated in the DNA damage response mechanisms by promoting repair of single-strand breaks and base excision sites. Depletion of the activity of this protein is regarded as a possible anticancer strategy [104,105]. In particular, the inhibition of PARP-1 activity has been extensively studied as a very promising strategy in the treatment of ovarian cancer with BRCA genes mutations, leading to the clinical approval of olaparib in 2014 by the European Union and US Food and Drug Administration [105,106].

Another strategy to impair DNA metabolism involves inhibition of Topoisomerase I and II functioning. These are ubiquitous nuclear enzymes able to cleave the phosphodiester DNA bond for the regulation of its topology [107]. Compound **53** was demonstrated to act on multiple targets: aside from TrxR inhibition, PARP-1 and Topoisomerases I and II inhibitory activity were also observed [108,109]. The silver carbene showed preferential activity against cisplatin-resistant A2780 cells and caused cell death by apoptosis. Moreover, it selectively inhibited glycolysis in tumour cells and was in general significantly less active towards non-cancerous cells. *In vivo* anti-tumour activity against cells of intraperitoneally implanted hollow fibres was found, but no antiproliferative effects for subcutaneously implanted hollow fibres were highlighted. A very recent work reported that the two cytotoxic gold dicarbenes **54** and **55** elicited good Topoisomerases inhibition and impairment of microtubules dynamics [110]. Docking studies have been used to evaluate the ligand abilities of the investigated complexes towards Topoisomerase I, II and tubulin. Experiments confirmed that **54** and **55** can inhibit Toposiomerases and act as tubulin polimerization inhibitor and microtubule stabilising agent, respectively. It was also shown that **54** causes MDA-MB-231 epithelial, human breast cancer cell death through a ROS-dependent mitochondrial mediated intrinsic apoptotic pathway. Results of MDA-MB-231 cell cycle analysis after treatment with the same compound also pointed towards a mechanism of action involving inhibition of topoisomerases and tubulin polymerisation.

More recently, the gold(I) NHC compounds **56**-**62** have been tested for their antileishmanial activity and found to inhibit enzymes regarded as important for anticancer strategies such as thymidine phosphorylase, β-glucuronidase and xanthine oxidase [111]. Later investigations were extended to the gold(I) dicarbene **63** and gold(III) complexes of the type [gold(III)$(NHC)_2X_2]BF_4$ **64**-**71** [84]. The panel of carbenes has been tested for anti-inflammatory properties, inhibition of

enzymes involved in metabolism (α-glucosidase; β-glucuronidase) and antileishmanial activity. These compounds have usually high cytotoxicity and dependence on the NHC substituents is observed but general trends seem difficult to establish. It is interesting to notice that only the gold(III) complexes are α-glucosidase and β-glucuronidase inhibitors. Recently, a target identification approach was adopted for C^N^C cyclometalated gold(III) NHC complexes [112]. The chemical probe **72** was built from previously reported compounds [113]: one N-alkyl chain was functionalized with a diazirine unit able to bind to interacting proteins upon irradiation; the other alkyl chain had a clickable alkynyl moiety to bind to a fluorescent reporter *in vitro*. This strategy allowed the identification of six bound intracellular proteins regarded as anticancer targets, namely mitochondria heat shock protein (HSP60), vimentin (VIM), nucleoside diphosphate kinase A (NDKA), nucleophosmin (NPM), nuclease-sensitive element-binding protein (Y box binding protein, YB-1), and peroxiredoxin 1 (PRDX1). The most active unfunctionalized compound **73** was found to compete with the probe for the binding to these proteins. Moreover, it was employed to demonstrate that the function of HSP60, VIM, NPM and YB-1 in HeLa cells was disrupted, justifying the observed anti-cancer effects, and confirming the consistency of the identified multiple targets.

In light of the correlation discovered between inflammation and cancer, the dinuclear silver complex **74** was investigated for its antiproliferative activity against prostate cancer cells and anti-inflammatory properties [114]. The complex is more cytotoxic towards HCT116 human colon cancer cells than human fibroblasts or prostate cancer cells and triggers apoptosis through the mitochondrial-dependent intrinsic pathway. Additionally, it suppresses the production of pro-inflammatory proteins such as IL-1 and TNF-α in human macrophages and inhibited cyclooxygenases (COX) in cell-free assays.

4. Nucleic acids as targets

4.1 Duplex DNA

Since the early studies on cisplatin, DNA has been considered a primary target for metal anticancer drugs [115]. For cisplatin the DNA binding type is covalent, it alters DNA functioning and replication, leading to cell death [116]. Similarly to cisplatin and other platinum-based chemotherapeutic agents, some NHCs can covalently bind to DNA [117]. Still, to the best of our knowledge, detailed studies on the binding of coinage NHCs to DNA are not abundant. Usually, group 11 complexes binding to DNA is mediated by non-covalent interactions and the focus of this paragraph will be on this type of binding. Non-covalent binding of metal NHCs with natural double-stranded DNA can be classified into two major binding modes: groove binding and

intercalation [118]. Classical groove binders are crescent-shaped molecules whose shape matches with the groove curvature of the helix [119]. Conversely, intercalation involves the insertion of planar aromatic moieties between the nucleobases of DNA: as a result, the helix elongates and unwinds [120]. As already discussed above, silver(I) and gold(I) NHCs antiproliferative effects are commonly related to antimitochondrial and protein inhibiting activity. Most of the compounds reported here are engaging both genomic and non-genomic targets which may be significant for the anticancer properties. To drive the affinity towards polynucleotides, appropriate ligands may be engineered. If this is not the case and the molecular structure is not adequate, calf thymus DNA (ctDNA) binding test will accordingly show low affinity [121,122].

4.1.1 Silver NHCs The benzotriazole functionalized Ag(I)-NHC **75** (Figure10) was found to affect the migration pattern of pBR322 plasmid DNA and docking test suggested that this compound may accommodate in the DNA groove [123]. Despite this latter result means that groove-binding species deserve interest, intercalation is known to be the most effective non-covalent mode of action to produce DNA damage. The length of alkyl chain substituents and the presence of aromatic residues can have significant repercussions on parameters such as hydrophobicity and planarity and, thus, on DNA interaction and intercalation ability. Haque and co-workers worked on the Ag(I)NHC complexes **76**-**79** from benzimidazolium salts; they were found to bind *Aedes albopictus* DNA (AaDNA) *via* intercalation, being the compound **76** bearing an additional benzyl group the one which produces the higher helix elongation [124]. A similar trend was found by the same research group by testing a series of new nitrile-functionalized benzimidazol-2-ylidenes and their Ag(I) and Hg(II) complexes. The most effective complexes, which intercalate into *Escherichia coli* genomic DNA (gDNA), are the mono and dinuclear benzyl-containing Ag(I) compounds **80** and **81**; **81** also efficiently cleaved supercoiled DNA form into linear form in the presence and absence of oxidizing agent [125]. The same holds for a series of mononuclear and binuclear Ag(I)NHC hexafluorophosphate complexes with complex **82** (the most extended one as for aromatic system) which exhibited the stronger Aa-DNA intercalation and the higher larvicidal effect [126].

4.1.2 Gold NHCs However, the addition of intercalating moieties do not always produce the desired effect: the acridine-decorated cyclometalated gold(III)-NHCs **83** showed high levels of ctDNA stabilisation suggestive of intercalation; on the other hand, the lack of a correlation between the cytotoxicity data and the interaction with double stranded DNA may suggest that the addition of the acridine moiety onto this scaffold did not result in improved DNA targeting [127].

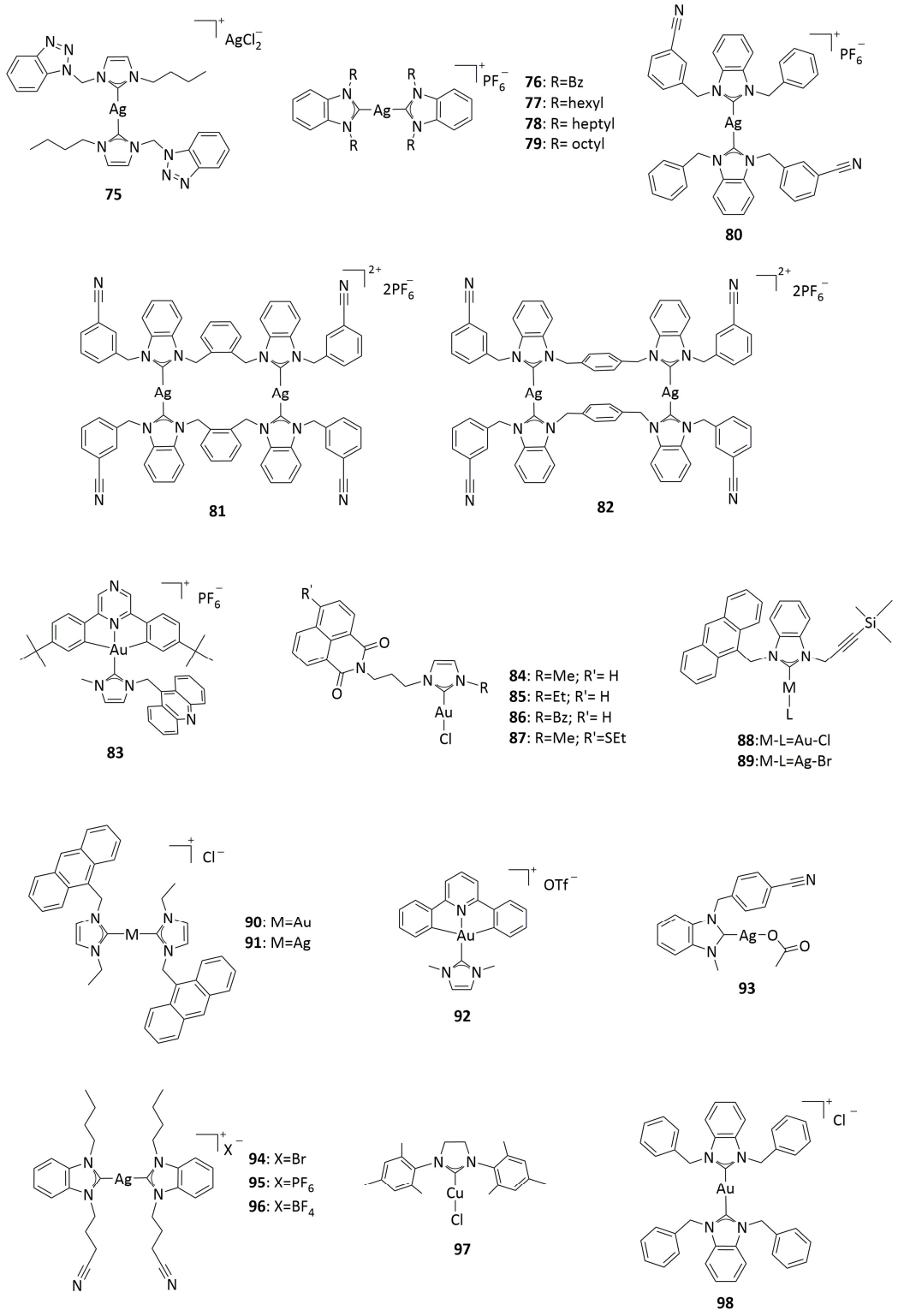

Figure 10. Chemical structures of coinage metal NHCs directed to duplex DNA.

On the whole, DNA may not be the only target for these compounds. In 2014, Ott and co-workers reported the design of bifunctional gold(I) NHCs able to interact with both TrxR and DNA [128]. This was achieved through functionalization of the NHC with naphtalimide moieties (compounds **84**-**87**), a well-known class of intercalating agents also investigated as potential anticancer drugs.

Complexes **84** and **87** are submicromolar TrxR inhibitors and efficient intercalators which stabilise the DNA double helix. Increased cytotoxicity against HT-29 colon adenocarcinoma and MCF-7 breast cancer cells was achieved for these series compared to the imidazolium salt precursors.

4.1.3 Comparison between silver and gold DNA binding modes Some of us recently investigated the DNA binding features of the anthracenyl functionalized gold(I) and silver(I) NHCs **88**-**89** together with their TrxR inhibition [129]. Interestingly, he carbenes differ in their DNA binding features: the gold(I) NHC **88** appears to interact mainly with the groove, while the silver(I) carbene **89** is a good intercalator, strongly stabilising the DNA double helix. Moreover, the silver carbene is a more potent TrxR inhibitor and displays an increased cellular uptake if compared to the gold analogue and cisplatin. However, the lack of significantly increased cytotoxicity may indicate that inactivation mechanisms occurring inside the cells. Fluorescence microscopy and cellular distribution studies corroborate the idea that both DNA and TrxR could play a role in the observed antiproliferative activity. On the basis of these promising results, gold(I) and silver(I) complexes of bis(1-(anthracen-9-ylmethyl)-3-ethylimidazol-2-ylidene) (**90**, **91**) were analysed in their ability to interact with biosubstrates [130]. A multi-technique study confirmed the significant differences in the binding features between Ag(I) and Au(I) compounds. The gold complex **90** covalently binds BSA but is scarcely interacting with polynucleotides and G-quadruplexes. Conversely, for the silver analogue **91** the binding to BSA is weak but it strongly/selectively interacts with double strands by a complex mechanism where intercalation plays the major role, but groove binding is also operative. Che and co-workers demonstrated that the pincer C^N^C 2,6-diphenyl-pyridine ligand led to very stable gold(III) NHCs, not undergoing reduction even in the presence of reducing thiols (see compound **92**) [131]. It was also shown that not only the gold(III) NHC **92** was an intercalating agent, but acted as a Topoisomerase poison. Indeed, it could stabilise the adduct between DNA and Topoisomerase I hampering its capacity to relax supercoiled DNA and could induce Topo I-mediated DNA strand breaks [132]. The compound also displayed promising *in vitro* and *in vivo* anticancer activity. Tacke and colleagues developed the silver acetate NHC **93** which was found to be a DNA and BSA binder able to overcome chemotherapeutic drug-induced resistance; unfortunately, when tested *in vivo*, it showed high toxicity and no useful therapeutic range was found [133].

4.2 RNA binding

Note that, among the already limited literature on mechanistic studies for silver and gold NHCs binding to polynucleotides, DNA is the only considered species. To the best of our knowledge RNA is neglected, even if also this nucleic acid may play a crucial role in anticancer strategies

[134,135]. There are only a few exceptions. Some tests by the group of Haque on the interaction with a plasmid DNA/RNA mixture of Ag(I)-NHCs **94**-**96** by electrophoresis are available, which seem to indicate that the interaction does indeed occur [136]. Also, in our recent work on **90** and **91** already cited above, we also analysed their binding to the synthetic RNA polynucleotides poly(rA), poly(rA)poly(rU) and poly(rA)2poly(rU) as representative models for single-, double- and triple-stranded helices [130] demonstrating that the Ag(I)-NHC shows high affinity for RNAs but in the double-strand form only.

4.3 Copper NHCs and DNA fragmentation

Some metal complexes can act as artificial nucleases able to cleave the phosphodiester bond of DNA causing its fragmentation and degradation [137–139]. Compound **97**, one of the few copper(I) NHCs investigated for their anticancer potentialities, was cytotoxic in the submicromolar range against various cancer cell lines and could convert the supercoiled plasmid pcDNA4TO into its open circular form under aerobic conditions [140]. Additional experiments under suitable conditions indicate that the complex acts as a Fenton-type reagent activating oxygen with the formation of hydrogen peroxide. Notably, the nuclease activity was not observed for the corresponding homoleptic dicarbene nor silver(I) or palladium(II) NHCs, highlighting the importance of the copper centre peculiar redox properties. Srivastava and co-workers reported some gel electrophoresis experiments showing that gold(I) imidazol- and benzimidazole-ylidenes with aromatic substituents were able to cause fragmentation of DNA extracted from U373 glioblastoma cell line [141]. Compound **98**, the only with a benzimidazol-ylidene ligand, was the most effective. The already mentioned complexes **76**-**79** also displayed DNA cleaving features as shown by gel electrophoresis experiments with plasmid DNA [124].

4.4 G-quadruplexes

G-quadruplexes (G4) are non-canonical DNA (or RNA) secondary structures characterized by guanine tetrads held together by hydrogen bonds. Their conformations can be classified according to the relative orientations of the four strands involved in the G4 and to the conformation of the residues not involved in the tetrad [142]. These conformations are enriched in key regions of the genome including telomeres and regulatory regions [143–145]. As knowledge on these secondary structures is gained and progress in mapping their location in the genome proceeds, many reports also revealed that the ability to stabilise G4s could be advantageously exploited for the development of compounds with improved antitumor activity [145,146]. For these reasons, many efforts have been paid for the development of G4 stabilisers or of species able to induce

G4 folding. In this sense, good G4 binders should feature extended planar aromatic moieties able to stack on the external G-tetrad and charged structures together with H-bond acceptors [147]. The metal centre acts as a charge withdrawing group enhancing the π interaction and can substitute monovalent cations bound to the nucleotide. There are only a few examples of covalent bound metal complexes, whereas in most cases the metal centre plays a structural role [148,149]. Among the many compounds synthesized, two organic G4 binders are currently in clinical trials for the treatment of human cancers [150,151]. It is relevant to mention that stabilising telomeric G4s was related to antitelomerase activity [147]. The enzyme telomerase is active in most of the tumour cells and, being responsible for the elongation of telomeric regions, plays an important role in the indefinite proliferation of cancer cells. Interestingly, it was pointed out that also auranofin attenuates the activity of telomerase in selected breast cancer cells through a ROS dependent pathway [152]. Casini and co-workers reported on the antiproliferative activity and G4 binding features of a series of caffeine-based and benzimidazolylidene NHC complexes including **99**-**105** (Figure 11) [153]. The most interesting ones are **99** and **105**. Nonetheless, **105** is poorly selective concerning its potent antiproliferative activity against cancer and healthy cells and its strong interaction with DNA irrespective of its secondary structure. On the other hand, the xanthine dicarbene **99** (Figure 12) exhibits strong and selective stabilisation of G4 also in the presence of an excess of duplex DNA [153,154]. Moreover, it discriminated between different cancer cells, and, despite its cytotoxicity in the micromolar range towards ovarian cancer cells, toxic effects in kidney and liver tissues were observed only after incubation with a concentration of **99** as high as 100 μM [153].

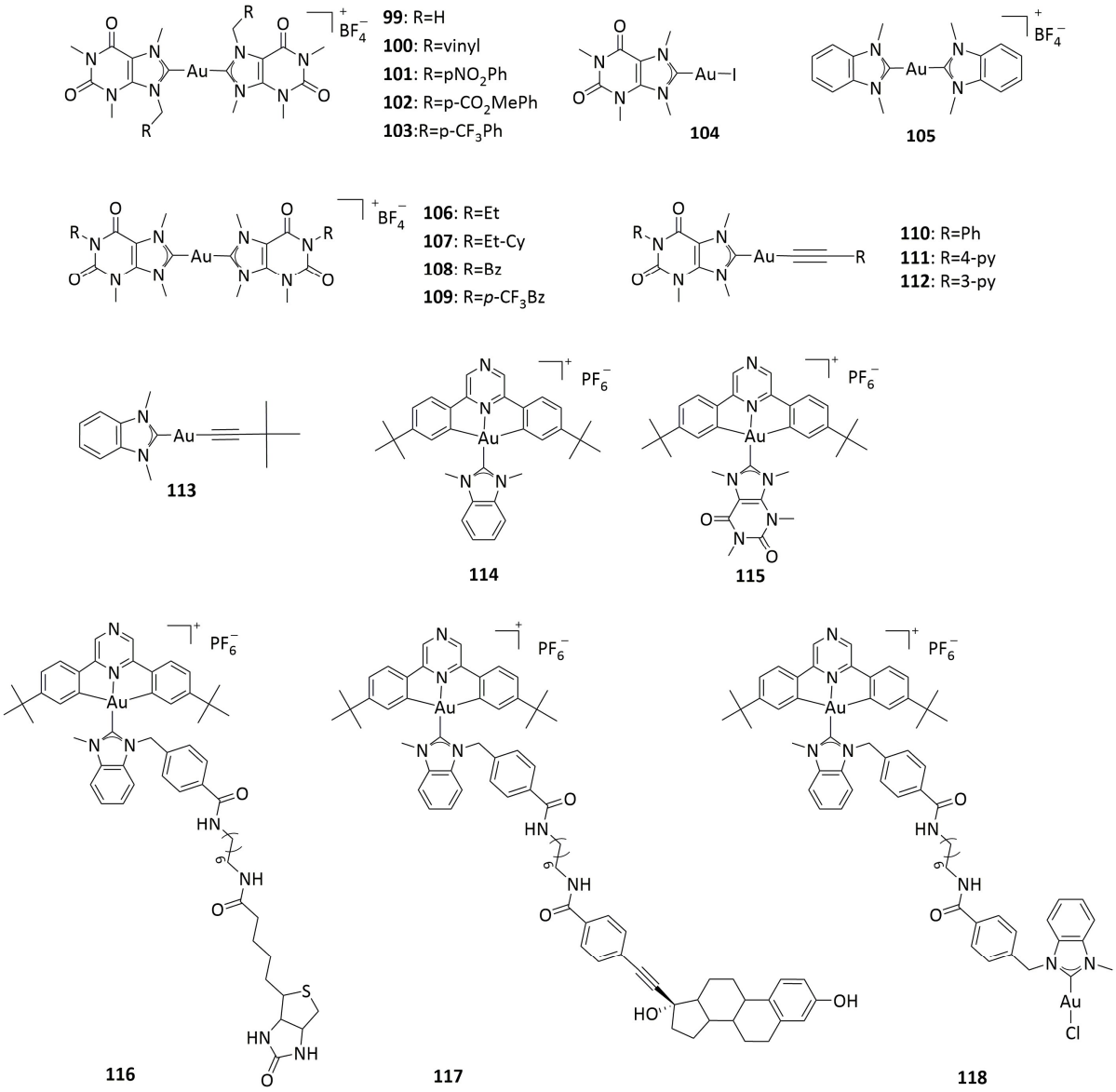

Figure 11. Chemical structures of metal NHCs directed to G-quadruplex DNA.

The gold dicarbene was crystallized with the telomeric G4 Tel23 leading to the resolution of the first crystal structure of this type (Figure 12a) [155]. Up to three gold complexes are bound to the telomeric structure with the gold atom at a short distance from nitrogen or carbon of the pyrimidine ring of the guanine (Figure 12b). Investigations in solution revealed that the binding interaction takes place through multiple cooperative reaction steps and the complex induced a transition in the quadruplex conformation from hybrid to antiparallel which is quantitative at gold-to-DNA molar ratios higher than 3 [156]. Also, a preformed adduct **99**/Tel23 is able to inhibit the activity of telomerase in a cell-free assay. A recent update consisted in the comparative investigation of the binding modes and free energy landscapes of **99** and **104** with two selected human G4

sequences through combined metadynamics and fluorescence resonance energy transfer (FRET) melting experiments. The theoretical results are in line with experimentally-derived data and can be important for the rational design of G4 binders [157]. A subsequent study on the similar compounds **106**-**109** allowed drawing of some structure-activity relationships [158]: substitution of the N1 nitrogen of the 9-methylcaffeine ring with more sterically demanding groups negatively affected G4 stabilisation activity being still **99** the most effective of the series. However, complexes **106** and **108** still exhibited selectivity for G4s over DNA double helix. On the other hand, the more sterically hindered complexes **107** and **108** acquired cytotoxicity towards the investigated cell lines which were not sensitive to **99** or **106**. Substitution of the second NHC ligand with an alkynyl to obtain compounds **110**-**112** resulted in a loss of G4 stabilisation. Among another series of alkynyl NHC, complex **113** was the only one exhibiting a significant stabilisation of hTelo and C-Kit1 (a human telomeric and promoter sequence respectively) [159]. However, the effect was still lower than the reference compound **99**.

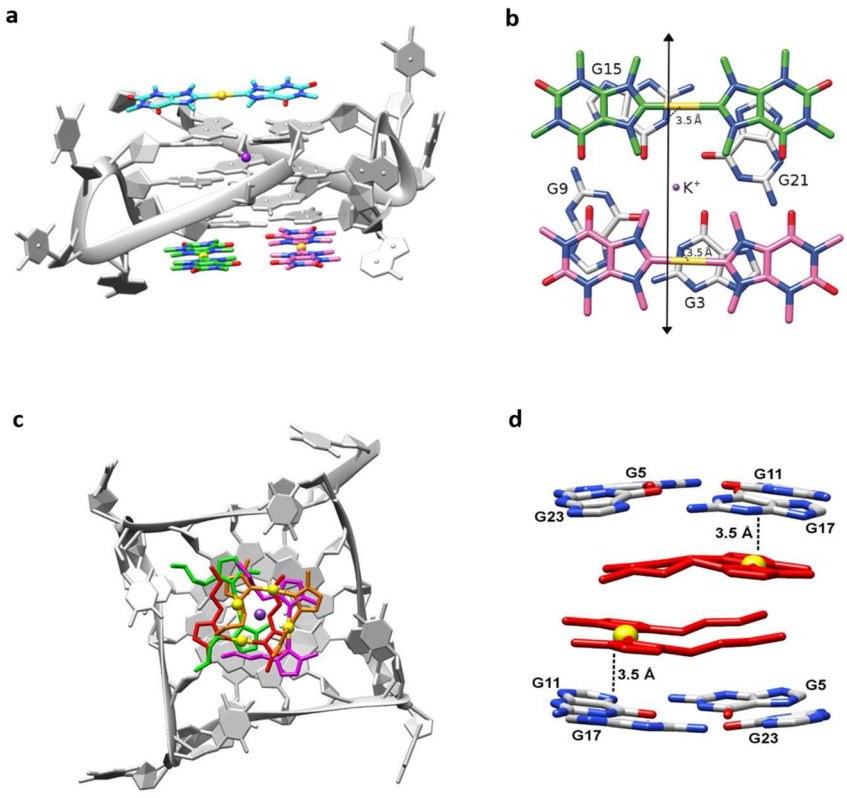

Figure 12. a) Representation of the crystal structure of **99/**Tel23, reproduced from PDB 5CCW, see also reference [155]; b) top view of **99/**Tel23 5-5' binding site reprinted with permission from ref [155]; c) top view of the 3'-end guanine tetrad of the Tel24 G4 with the stacked **25** complex spread over four crystallographic positions; d) detail of the binding site of **25**/Tel24 between two stacked Tel24 molecules, only one crystallographic position is shown. Potassium ions are shown in purple.

A recent work by some of us provided structural information on the adduct between the already mentioned dicarbene **25** and a telomeric G4 [160]. ESI-MS, circular dichroism (CD) and UV-Vis measurements revealed that, at variance, with the case of the caffeine NHC **99** the binding stoichiometry is 1 with no observed significant conformational changes or G4 stabilising effects. The crystal structure of the **25**/Tel24 adduct was solved thanks to the combination of x-ray diffraction analysis and quantum mechanics/molecular mechanics (QM/MM) level of theoretical calculations (Figure 12c). Overall, results suggest an entropy-driven modest interaction, characterized by a high degree of freedom of the complex in the binding site. Probably, the steric demanding alkylic substituents of the NHC hamper the formation of adducts with higher gold-to-G4 molar ratios. Notably, the position of the gold atom at a short distance from the N1 guanine nitrogen recalls what already found in the crystal structure obtained for Tel23 adduct with **99** (see Figure 12 panels b and d). This recurrent binding feature could be important for the rational design of Au-NHC G4 binders.

Bochmann and coworkers reported the preparation of pyrazine based C^N^C Au(III) complexes including the two compounds **114** and **115** with NHC ligands [161]. Compound **114** showed the most potent antiproliferative activity against various cancer cell lines and an increased uptake if compared to **115**. Remarkably, the whole structure was retained for at least 6 days in the presence of GSH, with no observed reduction or NHC hydrolysis, suggesting that its biological activity is due to non-covalent supramolecular interactions. Both carbenes are strong DNA G4s and i-motifs stabilisers and, to a lower extent, also double-stranded DNA stability is increased upon binding. Notably, this is the first report of gold compounds/i-motifs interaction. Moreover, **114** is the first compound whose ability to stabilise MDM2-p53 protein-protein interaction was evidenced. Further developments involved the preparation of dinuclear bioconjugate species [162]. While bioconjugation with biotin or 17α-ethynylestradiol as in compounds **116** and **117** increased cellular uptake, it diminished the ability of G4 stabilisation and was accompanied by a lower antiproliferative activity against cancer cells. On the other hand, the presence of a second metal centre (compound **118**) did not dramatically impact the affinity for G4s but negatively affected cellular uptake.

5. Conclusions

Given the drawbacks of cisplatin and following the continuous search of more effective and less toxic drugs, metal N-heterocyclic carbenes have attracted growing interest among the scientific community and several papers highlight their potential as new anticancer compounds. In our

opinion, the main peculiar feature which makes them extremely attractive is represented by their multimodal action. In this review, only focused on the main coinage metal-NHCs, the most important modes-of-action are presented and discussed, involving mitochondria, enzymes belonging to the cellular redox cascade, and nucleic acids.

Mainly, beyond the nature of the metal centre and its characteristic reactivity, the overall chemical structure can affect the biological behaviour of each metal-NHC class, making them for example more prone to penetrate the mitochondrial membrane instead of reacting with nucleic acids. In fact, the activity on mitochondria is strongly related to the interaction with the outer membrane of this biosubstrate. Therefore, the correct balance between hydrophobicity and hydrophilicity needs to be respected and fulfilment of optimal $LogP_{ow}$ values should be taken into account [66] jointly to the evaluation of the net charge of the molecule. To this regard, neutral molecules were found to target mitochondria well [68], but positively charged species seem to be more selective for cancer cells [49]. Charged and lipophilic complexes may show an increased mitochondrial and cellular accumulation which explained their more potent cytotoxicity compared to the neutral complexes. On the other hand, TrxR inhibition efficiency seems to be directly correlated with the presence of labile ligands on the molecule [75,92,93]. Also, a labile ligand favours in the case of gold carbenes the binding to the selenocysteine with extensive metalation degree [76].

Although organometallic NHCs are not known as molecules which show high affinity for nucleic acids, a rationale design with appropriate ligands, i.e. extended aromatic/planar residues could be the key to trigger their reactivity also against the genomic targets. For example, recent proof-of-principle studies on metal NHCs showed that this class of compounds is able to target several canonical and non-canonical DNA secondary structures. The intercalative binding with the double-stranded natural DNA helix [128,132] or favouring the sitting-atop binding to G-tetrads (in the case of G4s) favoured by geometrically constrained molecules are two main examples of such reactivity [153,155].

From the point of view of the metal centres, a different behaviour of Au(III) complexes from Au(I) ones is only observed when the Au(III) centre is stabilized by cyclometalated ligands. In this case, Au(III) compounds will exert their activity through supramolecular interactions more than through ligand exchange reactions [132,163]. When the reactivity of Ag(I)NHCs involves ligand exchange reactions this becomes comparable with the one of the Au(I) analogues but, depending on the binding group, Ag(I) species are more prone to metal centre loss [90]. On the other hand, significant variations in cellular responses are also observed between Au(I) and Ag(I) carbenes bearing the same NHC [72] and, in particular in the case of nucleic acids, different binding modes are observed depending on the metal centre [129,130]. Ag(I) NHCs are often cited as for their

antiproliferative properties, whilst the mechanistic aspects of their binding to the different biosubstrates is much less often analysed. Cu(II)NHCs have sometimes encountered stability issues but may still deserve high interest, also based on the Cu(II)/Cu(I) redox behaviour and endogenous nature of the metal centre.

The complete description of the mechanism responsible for the anticancer activity of metal complexes is a matter of major complexity. Even the mechanism of the forefather anticancer complex cisplatin is still largely unknown: the commonly accepted idea involving nuclear DNA platination as the only cause of apoptotic stimuli is now starting to be seen as an oversimplification [164]. Indeed, only a small part of platinum reaches the nucleus and the contribution of targets in other cellular compartments to the degree of responsiveness to the treatment are being taken into account [164,165]. As a matter of fact, there is now a paradigm shift from the idea of single-targeting to the one multitargeting metal complexes, and metal-NHCs behaviour perfectly fits with this “enlarged paradigm” where an important role has been devoted also to the reactivity with the cellular proteome. This idea applies both in the design of new complexes which are directed towards multifunctional components [166] and in the investigation of the effects of existing drugs which are found to be the results of the interplay of multiple activated pathways [167,168].

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LIST of ABBREVIATIONS

$\Delta\Psi_m$	transmembrane potential
AaDNA	Aedes albopictus DNA
AIF	apoptosis inducing factor
ATP	adenosine triphosphate
BIAM	biotinylated iodoacetamide (switch assay)
Bz	benzyl
BSA	bovine serum albumin
CD45	antigen also known as PTPRC
COX	cyclooxigenase
ctDNA	calf thymus DNA
Cys	cysteine
DFT	density-functional theory
DLC	delocalized lipophilic cation
ESI-MS	electrospray ionization-mass spectrometry
FAD	flavin adenine dinucleotide
G4	G-quadruplex, guanine-quadruplex (DNA form)
gDNA	Escherichia coli genomic DNA
GPX	glutathione peroxidase
GR	glutathione reductase
GSH	glutathione
HSP60	heat shock protein
ICP-MS	inductively coupled plasma mass spectrometry
IL-1	proinflammatory protein
$LogP_{ow}$	logarithm of the partition coefficient between octanol and water for a species
LYP	lymphoid tyrosine phosphatase
MMP	mitochondrial membrane permeabilization
MOMP	mitochondrial outer membrane permeabilization
MPT	membrane permeabilization transition
MS	mass spectrometry
NDKA	nucleoside diphosphate kinase A
NHC	N-heterocyclic carbenic ligand
NPM	nucleophosmin protein
PARP-1	zinc finger protein poly[ADP-ribose] polymerase-1
PEP	phosphoenolpyruvate
PET	positron emission tomography
Ph	phenyl
PRDX1	peroxiredoxin 1 protein
PTP	protein tyrosine phosphatase
PTP-PEST	protein tyrosine phosphatase belonging to the PEP family
PTPRC	protein tyrosine phosphatase, receptor type, C
ROS	reactive oxygen species
Sec	selenocysteine
TNF-α	proinflammatory protein
TrxR	thioredoxin reductase
VIM	vimentin protein
YB-1	nuclease-sensitive element binding protein, Y box binding protein

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