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Exploring the Potential of Metallodrugs as Chemotherapeutics for Triple Negative Breast Cancer

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Abstract

This review focuses on studies of coordination and organometallic compounds as potential chemotherapeutics against triple negative breast cancer (TNBC) which has one of the poorest prognoses and worst survival rates from all breast cancer types. At present, chemotherapy is still the standard of care for TNBC since only one type of targeted therapy has been recently developed. We will list references for metal-based compounds studied in TNBC cell lines and those of metal-specific reviews, but we will provide a detailed overview on compounds studied *in vivo* (mostly in mice models) and those compounds for which some preliminary mechanistic data was obtained (in TNBC cell lines and tumors) and/or for which bioactive ligands have been used. The main goal of this review is to highlight the most promising metal-based compounds with potential as chemotherapeutic agents in TNBC.

Graphical Abstract

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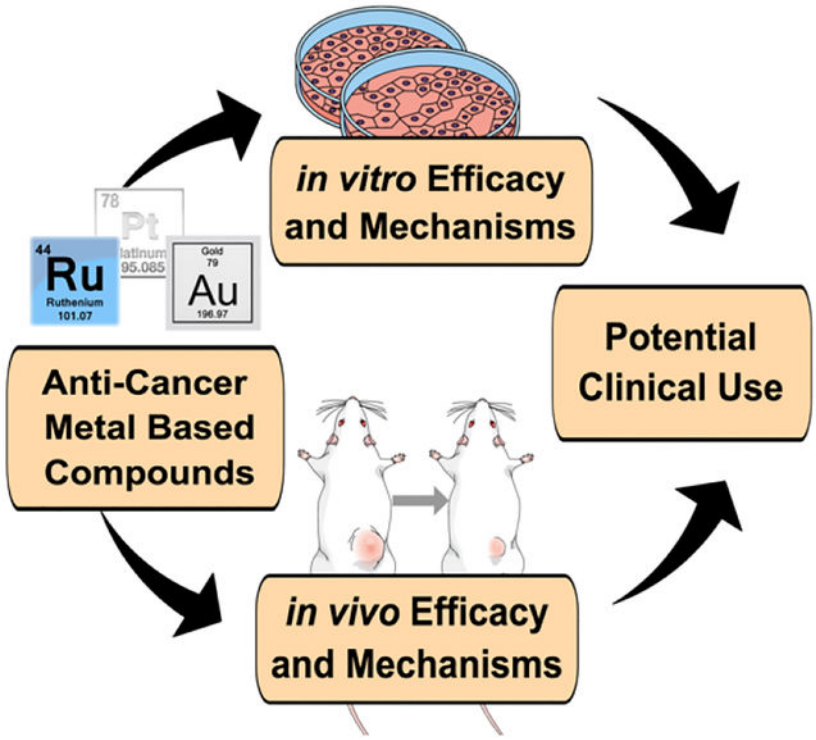
Declaration of Competing Interest

None.



Triple negative breast cancer (TNBC) has one of the poorest prognoses and worst survival rates for patients, with chemotherapy still the gold standard for treatment. This review collects information on all metallodrugs studied in TNBC cells and tumors, focusing on mechanistic and *in vivo* studies. An outlook towards TNBC metallodrugs with translational potential is provided.

Keywords

Inorganic Chemistry; Medicinal Chemistry; Triple Negative Breast Cancer (TNBC); Metallodrugs; Mechanisms

1. Introduction

Triple negative breast cancers (TNBC) are a major molecular subtype of breast cancer, including the basal–like type that does not express estrogen receptors, progesterone receptors and human epidermal growth factor receptor 2 (ER-/PR-/HER2-). TNBC has one of the poorest prognoses and worst survival rates for patients, accounting for about 15–20% of all breast cancers. Incidence and mortality of TNBC is often higher not only among younger women, but also disproportionately on women of African and Hispanic ancestry.[1–6] A key factor driving TNBC morbidity is its "molecular heterogeneity," described as "a lack of recurrent oncogenic driver alterations".[7] TNBCs are transcriptionally heterogeneous and can be grouped into four molecular sub-types (BL1, BL2, M, and LAR) "taking into consideration the contribution of transcripts from normal stromal and immune cells in the tumor environment".[7] Furthermore, the risk of recurrence during the first 5 years after diagnosis of (ER)-negative breast cancer is significantly greater than for those

patients with ER-positive tumors. Specific mutations in *BRCA1* and *BRCA2* human genes highly increase the risk of breast and other types of cancers in women, with a *BRCA1* mutation having an increased risk of developing TNBC.

TNBC patients are typically treated with a combination of surgery, chemotherapy and radiation. More than 500 clinical trials for TNBC have been completed (ca. 50) or are ongoing[8] evidencing an urgent need for novel TNBC treatments. Most of the clinical trials explore the combination of conventional chemotherapeutics such as Cisplatin, Oxaliplatin, Carboplatin, Paclitaxel, Gemcitabine, or Ixabepilone with either growth factor receptor inhibitors (Erlotinib), kinase inhibitors (Everolimus), antibodies (Bevacizumab, Cetuximab) or check-point inhibitors (Imprime).[8] Immunotherapy has so far proven a good option for some patients, but it has been noted that it should be implemented in the first-line setting of metastatic treatment, and in combination with other agents.[9] Unlike treatment for tumors overexpressing HER2, non-specific chemotherapy is the standard of care for TNBC as there is only one type of targeted therapy recently approved by FDA in 2020 (antibody-drug conjugate sacituzumab govitecan, Trodelvy, which targets the protein Trop-2).[10] Current chemotherapeutic strategies target DNA repair (platinum compounds), p53 (taxanes), or cell proliferation (anthracycline).[11] In the adjuvant (additional cancer treatment provided after primary one to avoid cancer recurrence) and neoadjuvant (treatment provided to shrink the tumor before surgery or radiation) therapy settings the most common regiment for moderate-to-high risk TNBC is sequential, dose-dense anthracycline-taxane (ACT) combination. However, not all the molecular subtypes of TNBC respond in the same way and thus the specific most effective adjuvant/neoadjuvant regimes still need to be determined for each patient. While forthcoming quantitative proteomics analysis integrated with genomics and drug sensitivity data[12] hold the potential to improve our understanding of the disease and lead to tailored therapies for TNBC, the prompt development of viable, affordable, less toxic but effective chemotherapeutic drugs for the different types of TNBC (to be used alone or in combination with other forms of therapy) would benefit a large number of patients in the immediate future.

Small molecules have been recently reviewed for the treatment of TNBC including a handful of organometallic compounds.[13] In this review, we will highlight recent selected triple negative breast cancer clinical trials based on FDA-approved platinum compounds. We will also report on preclinical studies for other platinum-based metal-based compounds (until September of 2020) that are discrete molecules (we will not include metallic nanoparticles collected elsewhere).[14] We will list the references for all compounds studied in TNBC cell lines (which include studies on MDA-MB 231, MDA-MB 468, MB157, BT-20, *BCRA1* gene mutation carrier L56Br-C1, and *BCRA1* defective HCC-1937) and those of metal-specific reviews. We will expand on compounds studied *in vivo* (mostly in mice models) and those compounds for which some preliminary mechanistic data was obtained (in TNBC cell lines and tumors) and/or bioactive ligands have been used. The main goal of this review is to highlight the most promising metal-based compounds with potential as chemotherapeutic agents in TNBC.

Platinum and Palladium Compounds

Currently, there is growing interest in the use of platinum agents in adjuvant therapy settings for the treatment of TNBC (especially for patients with *BRCA*-mutations with the direst prognosis,[15] and for some of the sub-types unresponsive to standard neoadjuvant regimes like ACT[7,11]). It is known that breast cancers with mutations in *BCRA1/2* are hypersensitive to DNA cross-linking agents due to impaired homologous recombination.[16] However, it needs to be considered that only a small fraction of TNBCs are *BCRA*-deficient.

Platinum (II) compounds

Platinum compounds have been studied in TNBC clinical trials (see structures of selected examples in Scheme 1A), typically in a neoadjuvant setting. There are currently 42 active platinum-based TNBC studies on clinicaltrial.gov, with 16 of these studies recruiting.[8] Que and co-workers have reported on a meta-analysis and systemic review of randomized control trials featuring platinum treatments in a recent review (2019).[17] The review evaluates the effect of platinum chemotherapy on the pathological complete response (pCR), which is the absence of residual invasive cancer and has been shown to improve overall survival in patients. While the review focuses on 11 trials, we will highlight the most successful ones. In general, advanced phase II/III clinical trials tend to feature carboplatin or cisplatin with a combination of other established anticancer drugs, such as docetaxel, paclitaxel, and gemcitabine.[17–22] The GeparSixto phase II trial (2014) reported on a 53.2% pCR with carboplatin in TNBC patients, versus a 36.9% pCR in patients that did not receive the platinum drug treatment.[18] The BrighTNess study (2018) is a double-blind randomized phase III trial investigating the neoadjuvant efficacy of carboplatin with PARP inhibitor veliparib, and described a 53% pCR, compared to 31% in the control paclitaxel with carboplatin and veliparib placebos.[19] The WSG-ADAPT-TN phase II clinical trial (2017) outlined a 45.9% pCR rate when patients were treated with a combination of Nab-paclitaxel and carboplatin.[20] The CBCSG006 phase III trial (2015) described that a cisplatin-gemcitabine regimen was more effective than a paclitaxel-gemcitabine treatment, although adverse drug-related effects were observed.[21] A phase II trial which involved neoadjuvant lobaplatin treatment, a third-generation platinum drug, reported on a 38.7% pCR with lobaplatin, docetaxel and epirubicin treatment, displaying higher efficacy than the treatment with just docetaxel and epirubicin at a 12.7% pCR.[22] These recent phase II/III trials demonstrate the potential efficacy of neoadjuvant platinum-based chemotherapy in TNBC.

A number of other platinum (II) compounds have been evaluated preclinically on breast cancer cell lines (including TNBC). We list here those for which the studies were focused on TNBC, and for which there were some mechanistic or *in vivo* studies.[23–31] Cationic or neutral platinum (II) derivatives with chelating (NN, CN) and labile ligands have been described. Cationic platinum (II) derivatives bearing only one labile ligand and incorporating imidazoles (like **1** in Scheme 1.B. reported by Rimoldi and co-workers) were found to be more effective than cisplatin in the TNBC cell line MDA-MB-231 (IC_{50} ca 62 μM, 48 h) by a mode of action different to that of cisplatin.[23] Compound **1** did not seem to interact with cellular transporters involved in cisplatin uptake (such as CTR-1, ATP7 and ATP7b), did

not reach nuclear DNA, and interfered with the progression of the G2/M phase of the cell cycle (while cisplatin increases the percentage of cells in the S phase). It was hypothesized that **1** may exert its cytotoxic effect via a p53-independent activation of the mitochondrial pathway.[23]

A cationic cyclometallated (CN) derivative containing an iminophosphorane ligand prepared in our laboratory (**2 Pt-IM**, Scheme 1.B.) was especially cytotoxic to the MDA-MB-231 cell line (0.84 ± 0.29 μM, 24 h).[24] This compound was found to be mainly caspase dependent at short incubation times, suggesting that the main mechanism of cell death for the compound is apoptosis. **2** does not interact with plasmid (pBR322) DNA or with calf-thymus DNA. Interestingly, permeability studies of **2** by two different assays, *in vitro* Caco-2 monolayers and a rat perfusion model, revealed a high permeability profile for this compound (comparable to that of metoprolol or caffeine) and an estimated oral fraction absorbed of 100%, which potentially makes it a good candidate for oral administration.[24] A series of hydrophobic organometallic derivatives such as most active **3b** and **3d** (Scheme 1.B.) reported by Ruiz and co-workers were found to be highly cytotoxic in MDA-MB-231 cell lines (values 4–6 μM, 48h).[25] The compounds were shown to activate apoptotic cascades, and to decrease ROS production without modifying the mitochondrial membrane potential. The compounds were also found to interact with DNA in a covalent manner, to display antiangiogenic effects *in vitro* and *in vivo* (on the chloroallantoic membrane, CAM of fertilized SPF-eggs), and to inhibit tubulin polymerization and destabilize cytoskeleton organization in 518A2 melanoma cells.[25]

A different strategy which has been used for a number of the compounds reported in this review, is the *coordination of bioactive ligands* to improve their biological profile. A series of platinum (II) derivatives based on diamines N,N,[26] or C,N[27–29] ligands containing the steroidal units estrone[26] or estradiol[26–29] have been reported with the aim to transport platinum (II) compounds into the cells. Estrone and estradiol play a very important role in the evolution of estrogen dependent breast cancers in more than 90% of cases. It was found that for these compounds, there was considerable cytotoxicity for both estrogen dependent ($ER\alpha^+$: MCF7) and independent (ERα: MDA-MB-468) human breast tumor cell lines (several times that of cisplatin).[26–29] For compounds like **4** (**VP-128**, Scheme 1.B.) containing 17β-estradiol, it was found that the estrogen receptor affinity (ERBA) was similar to that of 17 β-estradiol.[27–29] As expected, when tested *in vivo* in mice, the estradiol-platinum(II) hybrid **4** was more efficacious that cisplatin only for the xenograft models of ER+ breast cancer (MCF7)[26] highlighting the problem to target TNBC tumors which do not overexpress factors. The incorporation of a L-Tyrosine motif to N,N-chelating ligands rendered hybrids that were not cytotoxic to breast cancer cells.[30]

Very recently, Berners-Price, Farrell and co-workers have reported on the conformational modulation of iduronic acid-containing sulfated glycosaminoglycans (like the pentasaccharide Fondaparinux FPX, Scheme 1.B.) by a polynuclear platinum compound TriplatinNC (**TriPtNC**, compound **5** in Scheme 1.B.).[31] FPX is a valid model for the highly sulfated cell signaling molecule heparan sulfate (HS). The cleavage of HS in the extracellular matrix by human heparinase (hHPSE) is strongly linked to inflammation and cancer metastasis. In this report, the authors clearly demonstrated for the first time that

a small molecule may affect conformational changes on a HS oligosaccharide (**TriPtNC** drives the conformation of Iodo(2S) in FPX to the $^1C_4$ chair). This in turn (and coupled with results from previous studies on the interaction of TriPtNC with DNA)[32] allowed the authors to propose a dual role for this type of Pt complexes (cytotoxicity through DNA targeting and potential antimetastatic action through the above-mentioned interference with HS function).[31] Moreover, the authors reported on the effects of **TriPtNC** on bovine heparinase (bHPSE) in the TNBC cell line MDA-MB-231. A treatment of cells with this enzyme and pre-treatment with **TriPtNC** allows for a marked reduction in cell migration. Fluorescence cell-based assays indicated that **TriPtNC** and not cisplatin, blocks heparinase III cleavage on cell surface HS. Lastly, the antimetastatic activity of **TriPtNC** (**5**) *in vivo* was confirmed in a syngeneic 4T1 mouse-derived model of TNBC breast cancer metastasis to the lung by using a “mastectomy model”. There was a significant reduction of lung metastases and long-term survivors were identified.[31]

Platinum (IV) compounds

Platinum (IV) coordination compounds containing cisplatin and bioactive ligands in axial positions (such as COX- and PD-L1 inhibitors, RDA51-targenting moieties, vitamins, DNA-alkylating agents, tumor vascular disrupting agents and other drugs) which can enhance selectivity on cancer cells and/or achieve combined effects on different cellular compartments, have revealed themselves as efficacious in TNBC cell lines,[33–40] zebra fish,[37] and TNBC xenograft mice models.[38–40] The hypothesis is that these compounds act as prodrugs in blood plasma and get activated once inside the cells by reduction in the cellular milieu to Pt(II) active species and the bioactive ligand.

Conjugates of cisplatin like **6** (**Pt-IBu**, Scheme 2) containing cyclooxygenase inhibitors like the NSAID drugs (indomethacin and ibuprophen) reported by Hey-Hawkins and co-workers,[33] were found to be cytotoxic on the TNBC MDA-MB-231 cell line (with **6** displaying IC_{50} value in the nanomolar range 0.05 nM, 72 h). It was found, however, that the COX-2 inhibition was not directly responsible for the potency displayed.[33] Biotinylated Pt(IV) conjugates (like **7**, **Pt-Bio-1**, Scheme 2) reported by Guo, Wang and co-workers,[34] displayed a higher cytotoxicity than cisplatin on the MDA-MB-231 cell line (18 ± 2.7 μM, 48 h) while being more selective when studied on normal MCF-10A breast cell lines. The authors described the beneficial effect of having one unsubstituted axial ligand in terms of platination of the cell, and stronger interaction with DNA after reduction to Pt (II) species.[34] These authors also reported on cisplatin conjugates incorporating RAD51-targeting moieties (a protein crucial in homologous recombination that mediates the sensitivity of cancer cells to DNA-damaging agents) like artesunate (**8**, **Pt-Art2** in Scheme 2).[35]

The cytotoxicity of the compounds on *BCRA*-proficient cells like MDA-MB-231 cell line was higher than that of cisplatin (for **8**: 3.54 ± 1.76 μM, 72 h). It was demonstrated that these conjugates enter the cells efficiently and are reduced to Pt(II) precursors with concomitant release of artesunate. On ovarian cancer cell lines Caov3, the authors demonstrated the breakage of DNA double strands, a reduction of RDA51 expression and inhibition of RDA51 focci.[35]

Two different bioactive ligands have been coordinated in the axial positions of the same Pt(IV) center in compounds containing cisplatin, in order to induce cellular death and potentially overcome drug resistance. Conjugates like **9** (**PFL** in Scheme 2) containing tegafur (the prodrug of the antineoplastic agent 5-fluoroacyl 5-FU which is a thymidylate synthase inhibitor) and lonidamine (an indazole carboxylate that sensitize tumors when used in combination therapy with antineoplastic agents by triggering the mitochondrial pathway) were especially cytotoxic to the MDA-MB-231 cell line (2.0 ± 0.2 μM, 72 h) while being taken up into the cells efficiently.[36] Compound **9** induces cell cycle arrest in the S phase (like cisplatin), but mainly accumulates in the mitochondria. The exposure of MDA-MB-231 cell line to **9** induces mitochondrial swelling and decrease of mitochondrial membrane permeabilization (MMP). In addition, conjugate **9** was able to reduce ATP production, to inhibit mitochondrial respiration and to elevate the level of ROS production.[36] By way of western blot analysis, it was demonstrated that the treatment of the cells with **9** changed the expression levels of the Bcl-2 proteins (Bax and BCl-2) which led to the release of cytochrome c from mitochondria. Interestingly, in this report from Tan, Mao and co-workers, [36] a western blot analysis shows the formation of ternary complex of thymidylate synthase confirming the release of one of the axial ligands tegafur and its conversion to 5-FU inside the TNBC cells. An RNA sequence transcriptome analysis indicates that **8** is involved in DNA synthesis, metabolism and damage.[36]

A Pt(IV) conjugate containing a tumor vascular disrupting agent DMA = 5,6-dimethylxantthenone-4-acetic acid) and cisplatin (**10**, **PDMA** in Scheme 2) was recently reported by Guo, Wag and co-workers.[37] Compound **10** was found to be more cytotoxic to TNBC cell lines MDA-MB-231 (3.3 ± 0.4 μM, 72 h) than cisplatin. The axial ligands present in compound **10** increase its lipophilicity and cellular uptake. The compound was found to damage DNA (by increase of expression of γH2AX DNA damage marker) and to display antimigratory and antiangiogenic properties. The antiangiogenic properties of **10** were demonstrated *in vivo* in a Tg zebra fish model along with a lower toxicity in this model than cisplatin.[37]

Three examples on Pt(IV) compounds tested *in vivo* in TNBC xenograft Balc/C nude mice models bearing MDA-MB-231 tumors have been recently reported.[38–40] Conjugates based on cisplatin and one or two molecules of chlorambucil (a well-known glutathione (GSH) interacting agent) like compound **11** (**CLB-Pt-CLB**, Scheme 2) were shown to be highly cytotoxic (ca. 2–3 μM, 48 h) on MDA-MB-231 cell lines (displaying much stronger cytotoxicity than either monotherapy or combination of chlorambucil and cisplatin). The cellular uptake, apoptotic and antimigratory behavior and DNA damage (evaluated by COMET assay) was considerably increased for compound **11** with respect to cisplatin. Compound **11** inhibited tumor growth (59% after 12 days of treatment, 2 mg Pt per kg, every 72 hours) in mice showing comparable antitumor activity to cisplatin but with a better toxicity profile (mice did not lose weight for the group treated with **11** as opposed to weight loss exhibited by the group treated with cisplatin).[38] A slightly earlier report on compound **11** by Dhar and co-workers[41] showed high cytotoxicity on the NCI 60 cell-assay. The activity of **11** was further enhanced upon encapsulation in the hydrophobic core of polymeric nanoparticles (**11-NP**) and in nanoparticles modified

with mitochondria-targeted TPP cations to access mtDNA and mGHS (**11-NP-T**). Pt-adduct analysis in cisplatin-resistant A2780/CP70 demonstrated enhanced cellular uptake of **11** and its NP formulations, as well as greater Pt-mtDNA adduct formation for **11-NP-T**. The GHS levels in these cells were lower for **11-NP-T**. Mitochondrial metabolism studies indicated activity of **11-NP-T** to interrupt energy production via glucose, glutamine, and fatty acid pathways. Citrate synthase activity confirmed the mitochondrial activity of **11** and its nanoformulations.[41]

A small library of Pt(IV) conjugates based on cisplatin and chemosensitizer adjudin (ADD) (like compound **12**, **Pt-ADD** in Scheme 2) were developed.[39] These compounds can self-assemble via a nanoprecipitation method into nanoparticles of small particle size, uniform morphology, and ultra-high drug loading content (84.0–86.5%). The addition of 1,2-Distearolylsn-glycero-3-phosphoethynolamine-N-[methoxy(poly-ethylene-glycol)-2000] (DSPE-PEG) generated **Pt-ADD-PGE** nanoparticles with increased hydrophilicity and stability. The cytotoxicity of the compounds in MDA-MB-231 cell lines was high (e.g. for **12 Pt-ADD-PGE**, 2.4 ± 0.7 μM, 48 h). The cytotoxic effects were mainly attributed to two pathways *in vitro*, namely direct DNA injury and mitochondria associated apoptosis. Three PGE compounds of the series were evaluated *in vivo* in Blac/C nude mice bearing MDA-MB-231 tumors and they exhibited enhanced tumor inhibitor effect (cisplatin did not produce this effect). Interestingly, the combination of ADD with cisplatin did not significantly suppress the tumor growth but did decrease the survival of mice (due to high systemic toxicity).[39] Further studies with compound **12 Pt-ADD-PGE** showed it displays good retention in tumor without obvious liver and kidney damage. The tumor section of mice treated with these NPs displayed the largest necrotic area, lowest Ki67 expression, highest cleaved caspase-3 and γH2AX expression.[39]

Very recently the laboratories of Guo and Wang have reported on a compound of Pt(IV) containing cisplatin and the NSAID naproxen NPX (**DNP**, **13**) which exhibited high cytotoxicity, apoptotic, antimigratory, and anti-inflammatory properties superior to cisplatin and NPX (Figure 1).[40]

The IC_{50} values in MDA-MB-231 cell lines were in the nanomolar range (0.16 ± 0.01 μM, 48 h) and *in vivo* studies in Blac/C nude mice bearing MDA-MB-231 tumors showed potent antitumor activity (tumor growth inhibition of ca. 46%) and almost no systemic toxicity. It was demonstrated that **13** does not get reduced to Pt(II) species and that it does not interact with DNA. The cell cycle arrest induced by **13** is very different from that of cisplatin indicating a different mode of action. Detailed mechanistic studies showed that compound **13** downregulates the expression of COX-2 and PD-L1 *in vitro* and *in vivo*, inhibits the secretion of prostaglandin, reduces the expression of the protein BRD4 and phosphorylation of extracellular signal-regulated kinases ½ (Erk1/2) while blocking the oncogene c-Myc. Moreover, **13** intercalates into nuclear DNA to form a chimeric adduct (damaging DNA and upregulating the expression of γ-H2AX). Compound **13** act as a “whole” and not by releasing NPx and reducing Pt(IV) to Pt(II). It seems that multispecific compound **13** intervenes in inflammatory, immune, and metastatic processes in breast cancer.[40]

Palladium (II) Compounds

Palladium (II) compounds have been explored as anticancer agents (over >850 compounds since 1980)[42] with one compound, padeliprofin (TOOKAD® depicted in Scheme 3, designed for vascular-targeted photodynamic (VTP) therapy) approved for clinical use in patients with prostate cancer. While a large number of these compounds possess some challenging features (low solubility, high lability and a very fast ligand exchange rate compared to platinum analogs) they represent an alternative to classical platinum (II) derivatives, displaying cytotoxicity and a higher selectivity to cancerous cell lines. Diniz and co-workers reviewed the activity of palladium (II) compounds against triple negative breast cancer in 2019, listing 121 palladium (II) derivatives (no palladium (IV) compounds have been reported so far for TNBC studies).[43]

The compounds were classified in groups by similarity of their main ligands: a) ethyl diamine, b) biogenic polyamines, benzyl amines/imines, c) pydirine/pyrazole/imidazole/pyrrol/triazole, d) chloroquine/clotrimazole, e) phosphane, f) thioureas and g) thiosemicarbazones. The authors listed all the compounds including their IC_{50} values in TNBC (mostly MDA-MB-231, but also MDA-MB-468, BT-20, and L56Br-C1), their selectivity to normal cells (when known), as well as the possible target or mode of action.[43] The authors mentioned the impossibility of establishing an accurate SAR due to the variability in the cytotoxicity evaluation studies and the structural variety. They were, however, able to draw some conclusions[43] that we list here: (i) compounds comprising amines were shown to be predominantly DNA-damaging agents (covalent bonding of compounds to nucleophilic nitrogen atoms of the DNA bases (mainly purines); (ii) induction of apoptosis was reported for some of benzyl-amine/imine complexes while for pydirine/pyrazole/imidazole/pyrrol/triazole both apoptosis and necrosis were recorded; (iii) for a large number of complexes displaying promising antineoplastic properties [(S-containing complexes of thiourea or thiosemicarbazones] the mode of action at molecular level is still unknown; (iv) best choice of ligands for the Pd(II) compounds seem to be N-containing polydentante molecules and/or sulfur moieties which provide higher stability and may ensure intracellular stability; (v) a third of the compounds studied exhibit IC_{50} values between 0.1 and 5 μM (much superior to cisplatin); (vi) the moderate hydrophilicity of Pd(II) complexes points to an intravenous administration for the compounds developed so far; and (vii) a number of compounds have exhibited cancer selectivity towards TNBC.[43]

We include two other types of compounds (**14** and **15** in Scheme 3) not mentioned in this review, and interesting due to: a) their cytotoxicity on TNBC cells with enhanced apoptotic effects and phototoxic activity in the presence of visible light (**14**),[44] and b) forming a metallosupramolecular structure (compound **15**) that resulted highly cytotoxic on TNBC cells by membrane disruption through the helicate structure.[45]

Scheme 3 also depicts structures of two types of compounds (**16** and **17–19**) mentioned in the review,[43] that we consider more relevant as their mechanisms have been studied more in depth[46–53] including some *in vivo* studies on mice (but not on TBNC tumors).[49,50,52] The binuclear cyclometallated Pd(II) compound [Pd(C^N)Cl(dppe)] (**16**, **AJ-5**) was found to be highly cytotoxic to the TNBC MDA-MB-231 cell line (IC_{50} = 0.193 μM, 48 h) while

being less cytotoxic to normal cells.[46] It was found that compound **14** has *in vitro* activity against MCF7 derived stem-like cells, that induces DNA damage (inducing double strand breaks DSBs) leading to G1 cycle cell arrest (increased levels of p21 were found but not of p-53 suggesting a p53-independent mechanism).

Compound **16** induces intrinsic and extrinsic apoptosis, autophagy and inhibition of the mTOR pathway in breast cancer cells (cells treated with **16** displayed swollen mitochondria, and increase of organelles in autophagic vacuoles while inhibiting levels of p-mTOR and its direct substrate p70 S6 kinase accompanied by a dramatic increase in LC3II a marker for autophagy as well as an increase of cleaved PARP and Beclin1 involved in cytochrome c release from the mitochondria). The cells treated with **16** presented increased p-38 levels.[46] In a different study it was demonstrated that compound **16** inhibits melanoma growth in nude mice without any noticeable side-effects.[47]

Ulukaya and co-workers reported on a series of cationic palladium (II) compounds containing saccharinate and triamine ligands such bis(pyridinylmethyl)amine (bmpa)[48] or 2,2′:6′,2′′-terpyridine (terpy)[49–53] which were found effective on TNBC cell lines,[48–53] and in some cases, in mice models[49,50,53] (selected compounds **17**-**19** in Scheme 3). The compounds are apoptotic.[48–53] For compound **18**, there is DNA damage (induction of double stranded breaks and DNA fragmentation and modification of secondary structure) and increase of cleaved PARP, induction of caspase 3 and 7 activity and pyknotic nuclei.[49] For compound **19** the apoptosis was shown to proceed via DR4 and DR5 genes[50] and a more detailed biochemical and proteomic analysis of this compound demonstrated that the mechanism of action of this agent involves induction of ROS, DNA damage (majorly by formation of DSBs) and NHEJ was indicted as the possible mechanism of repair.[51] Compounds **18** and **19** appear to be cell-cycle non-specific (CCNS) drugs[52] and presented superior efficacy to cisplatin and comparable to paclitaxel *in vivo* on Balc/c mice in the allogeneic Ehrlich ascites models of cancer with only some temporary side effects.[49,50] Moreover, compound **19** was studied on a lung mouse (C57BL/6) mice model where this compound reduced tumor size at half dose of cisplatin exerting less liver damage.[53]

2. Gold, Silver and Copper Compounds

The activity of coinage metal derivatives against different diseases has been known for centuries. More recently, in addition to their well-known activity as anti-inflammatory, and antimicrobial agents, some coinage metal compounds like gold (I) Auranofin (Scheme 4) have been studied in clinical trials against non-small cell lung and ovarian cancer.[54] The activity of gold, silver and copper complexes against breast cancer was reviewed in 2012 by Biersack and co-workers[55] and a few examples of gold,[56–63] silver,[64–66] and copper[66–69] compounds with activity on TNBC were described. Here, we also include more recent articles on this topic for these metals.[70–74]

Gold (I) and Gold (III) Compounds

The activity of gold (I) compounds containing phosphane ligands against TNBC cells[53–59,70,71] was first reported by Berners-Price and co-workers.[56] They described the activity of cationic bis-chelated Au phosphane complexes like compounds **20** and **21** which

displayed activity against TNBC MDA-MB-468 in the nanomolar range (ca. 0.5 μM) while being very selective (IC_{50} in normal cells > 100 μM).[56] Compound **20** selectively induced apoptosis in MDA-MB-468 cells via the mitochondrial pathway (which involved membrane potential depolarization, depletion of glutathione pol and caspase-3 and capsase-9 activation with accumulation of **20** on the mitochondria). Compound **20** inhibited the activities of both thioredoxin and thioredoxin reductase (an effect more pronounced in the TNBC breast cancer cells).[57] Moreover, the subcellular distribution of gold in MDA-MB-231 cells for compound **21** was performed by NanoSIMS ion maps and by energy filtered transmission electron microscopy indicating that it was associated with sulfur-regions in the nucleus and cytoplasm. This fact supports the evidence for the mechanism of action of gold(I) compounds on the inhibition of thiol-containing protein families.[58] A series of neutral gold(I) compounds containing heterocyclic N-carbenes (like compound **22** in Scheme 4) derived from 4,5-diarylimidazoles were reported by Gust and co-workers.[60] The compounds were active against MDA-MB-231 cells (low micromolar range) while causing thioredoxin reductase inhibition in the nanomolar range but at much higher concentrations than that displayed by Auranofin. The authors dismissed TrRx as the main target along with DNA interactions, ER binding and inactivation of COX enzymes for compound **22** (although these experiments were not performed in TNBC cell lines).[60]

More recently, Ruiz and co-workers have reported on the modification of gold(I) compounds by coordination of bioactive ligands.[70,71] Cationic compounds like **[Au(ACRTU)$_2$]Cl** (**23** in Scheme 4) containing two or one DNA intercalator ligands (1-acridin-9-yl-3-methylthiourea) were described.[70] The compounds displayed IC_{50} values in the low micromolar range for MDA-MB-231 cells, antiangiogenic and antimigratory behavior and they were able to inhibit vasculogenic mimicry of these cells. The apoptotic behavior observed was caused by caspase 3 activation and not by activation of mitochondrial pathways. Cycle arrest occurred in the G2/M phase (like for other DNA damaging agents). The compounds presented a DNA-dependent mechanism of action as confirmed by their location in the nucleus (by confocal and transmission electronic microscopy). It was shown that the compounds intercalate in DNA and exhibit a dose-dependent response on topoisomerase I mediated unwinding.[70] In 2020, this group also reported on the coordination of a gold-phosphane ($AuPPh_3$) fragment to the FDA approved Erlotinib (which targets the epidermal growth factor and its receptor EGFR through inhibition of EGRF tyrosine kinase activity through an ATP-competitive binding of the kinase domain) to generate conjugate **24**.[71] Compound **24** displays cytotoxicity against MDA-MB-231 cells (68-fold higher than Erlotinib while still being selective when compared with normal cells) and apoptotic behavior (causing DNA damage and production of ROS). Interestingly, the cell death cycle involves mainly arrest is S and G2/M phases primarily (while Erlotinib bocks the G1/S transition and increases G1 cell population).[71]

Square-planar gold (III) compounds have been studied as anticancer agents for the past two decades due to the fact they are isoelectronic to cisplatin and other platinum (II) derivatives and mostly adopt a similar geometry. Stable coordination gold (III) compounds containing dithiocarbamates reported by Fregona and co-workers (like **[Au(DMDT)Br$_2$]**, **25**, Scheme 4) were shown to be cytotoxic to cancer cell lines resistant to cisplatin and acted in a fast

way, inhibiting DNA and RNA synthesis but with weaker interactions with DNA when compared with cisplatin.[75] Dou and co-workers continued studies on these derivatives and identified the proteasome as a primary target for compound **25** *in vitro* and *in vivo* (MDA-MBA231 cells and xenografts).[61,63] The apoptotic behavior of **25** was associated with inhibition of proteasomal activity (especially chymotrypsin-like activity). *In vivo* studies in female athymic nude mice bearing MDA-MB-231 tumors showed potent antitumor activity (50% tumor growth inhibition) and little systemic toxicity.[61,62]

In 2014, Fregona, Dou and co-workers reported on the modification of compounds like **25** by coordination of oligopeptides (like compounds **26a**, **AuD6** and **26b**, **AuD8**) aiming to improve their specificity and pharmacological profile by better uptake via peptide transporters.[73] They demonstrated that proteasome was the major target for the two compounds reported in MDA-MB-231 cell lines, and mice xenograft models. They also observed 53% of growth inhibition (with some animals even showing tumor shrinkage) for mice treated with a low dose (1 mg/kg per day for 27 days) with almost no systemic toxicity. *In vitro*, compound **26b** showed a degree of selectivity toward the β5 subunit chymotrypsin-like activity, and the compounds containing oligopeptides were about an order magnitude than the first-generation compounds (**25**).[73]

Che and co-workers reported on another family of gold (III) anticancer agents containing meso-tetraarylporphirin complexes that are stable in aqueous solutions and under physiological conditions.[63,76–80] One of these derivatives, compound **27** (**[Au(TPPP-OH)]Cl** or **gold-2a** in Scheme 4) was highly cytotoxic against MDA-MB-231 cells (ca 1 nM, 48 hours) and *in vivo*. In female athymic nude mice it was demonstrated that the administration of two bolus of **27** (15 mg/kg) by intraductal injection into the MDA-MB-231 tumor xenograft resulted in a complete remission in 50% of the animals (which remained in a tumor-free status until day 25 with much lower toxicity than cisplatin).[63] Experimental and computational data demonstrated that the effects are due (at least in part) to the attenuation of Wnt/β-catening signaling via inhibition of class I histone deacetylase (HDAC) activity.[63] Subsequent reports on related gold (III) porphyrins (like [Au(TPPP)]Cl without the hydroxyl substituent of compound **27**) on different cancer cell lines and tumor xenograft models, indicated that these compounds are highly active, apoptotic and target a mitochondrial chaperone protein, most likely through noncovalent interactions.[76–79] More recently,[80] a member of this family [AuMesoIX] with an activated porphyrin scaffold, has been shown to modify reactive cysteine residues and inhibit targets including thioredoxin, peroxiredoxin, and deubiquitinases by activation of the meso-carbon atom of the porphyrin ring by the gold(III) center and aromatic substitution with thiols. It was demonstrated that this compound generated oxidative stress-mediated cytotoxicity, gold-bound sulfur rich protein aggregates and accumulation of ubiquitinated proteins while being very effective in an ovarian xenograft cancer mice model.[80] A related highly stable gold (III) compound with a corrole core reported by Gross and co-workers,[72] was found to be a little more active on MDA-MB-231 cells than cisplatin and its low affinity for HSA was demonstrated. It was mentioned that this compound was more hydrophilic than the gold (III) porphyrins described above.[72]

Silver (I), Copper (I), and Copper (II) Compounds

Silver (I) compounds containing N-heterocyclic carbene ligands derived from 4,5-dichloro-1H-imidazole or 4,5-diarylimidazole have been described to display moderate cytotoxicity on TNBC cells MB157,[64] and MDA-MB-231[65] respectively. In the case of the report by Gust and co-workers,[65] targets like DNA, ER and COX enzymes were excluded. The activity of compounds containing bioactive acetylsalicyclic acid AAS fragments (coordinated to the silver(I) or copper (I) centers on the triple bond of the ligand, compounds **28**, **Di-ASSS-But-Ag** and **29**, **Di-ASSS-But-Cu** in Scheme 4) was studied against MDA-MB-231 cell lines.[66] Only silver compounds like **28** resulted cytotoxic (IC_{50} ca 5.0 μM, 72 h). Both silver and copper compounds displayed inhibitory effects on COX-1 and COX-2 enzymes but not as strongly as the AAS.[66]

Dinuclear copper (II) compounds containing isoxazole-derived aroylhydrazones displayed cytotoxicity on MDA-MB-231 (sub-micromolar range) and the interactions of these compounds with calf-thymus DNA was demonstrated.[74] More effective copper (II) compounds with dithiocarbamate ligands were reported by Dou and co-workers.[67–69] The addition of $CuCl_2$ to Disulfiram (DSF) a drug used for the treatment of alcoholism (and with antitumor and chemosensitizing activities) generates a DSF-copper complex (**30** in Scheme 4) that is cytotoxic on MDA-MB-231 cells, highly selective when compared to MCF-10A breast cells, and like gold (III) compound **25**, inhibits the proteasomal activity in these cells before inducing apoptosis.[67] Moreover, DSF by itself induces inhibition of proteasome and induction of apoptosis in copper-enriched MDA-MB-231 cells. DSF was administered by itself in female nude mice bearing MDA-MB-231 tumors (known to contain increased cellular copper levels) leading to tumor growth inhibition (by 74%) associated with *in vivo* proteasome inhibition and apoptosis induction. It was demonstrated that DFS can target tumor cellular copper to exert cytotoxic and apoptotic effects *in vivo*.[67] A later report by this group on discrete Cu(II), and Zn(II) compounds containing different dithiocarbamates (PyDT)[68] and (EDTC)[69] demonstrated the cytotoxic effects of these compounds on MDA-MB-231 cells associated to the inhibition of proteasome (either cellular 26S proteasome with JAMM domain-containing 19S particles[68,69] or purified 20S proteasome (without 19S particles containing JAMM domain).[69]

3. Osmium, Ruthenium and Iron Compounds

Complexes containing group 8 metals osmium, ruthenium and iron have shown promising anticancer properties and have been heavily studied during the past 20+ years.[81,82] Ruthenium compounds have emerged as attractive alternatives to cisplatin and as potential follow-up agents to conventional platinum-based drugs, specifically targeting metastasized solid tumors and cisplatin-resistant tumors and endowed with different mechanisms of action.[81,83] Three Ru(III) derivatives, NAMI-A and KP1019/KP1339 (Scheme 5) entered clinical trials for colorectal cancer,[84,85] while a Ru(II)-based photosensitizer (TLD-1433) has recently been fast-tracked by FDA in phase II clinical trials for treating non-muscle invasive bladder cancer (Scheme 5).[86]

Osmium (II) compounds

The octahedral configuration and preference for higher oxidation states for osmium favors interactions with diverse cellular targets such as proteins and DNA, with the possibility of redox modulation.[87] Cytotoxic effects and an apoptotic mechanism of cell death have been described for osmium-based carbonyl cluster complexes in TNBC (MDA-MB-231) cell lines.[88]

Brabec and co-workers developed a half-sandwich osmium (II) compound containing bathophenanthroline attached to the metabolic modulator dichloroacetate (dca), compound **31** (**Os-dca**, Scheme 5).[89] Compound **31** displayed cytotoxic effects in MDA-MB-231 cells (IC_{50} = 0.5 ± 0.02 μM, 72h) and was discovered to significantly decrease lactate production, suggesting glycolytic inhibition as a mechanism of action. A significant reduction in the protein expression of aquaporin 5, a water channel implicated in cancer proliferation, was also observed in MDA MB-231 cells treated with **Os-dca** (**31**). Enhanced hydrolytic ability of the metal (Os) allows for easy release of the dca ligand in water-containing solvents and an improved pharmacological profile.[89]

Ruthenium Compounds

As stated above, ruthenium compounds have become of interest as anticancer agents due to their ability to specifically target metastasized solid tumors and cisplatin-resistant tumors. It is well-known that the ruthenium metal provides access to multiple oxidation states and increased selectivity to tumor site due to interactions with blood transporter proteins.[90] A excellent recent review by Castonguay and co-workers on ruthenium compounds as potential breast cancer chemotherapeutics (including TNBC) collected the varied approaches to ruthenium-based drug design.[91] We will highlight here the compounds studied on TNBC cell lines and i*n vivo* models, as well as some that have been reported since its publication.

Ruthenium (II) Compounds

A large number of ruthenium (II) complexes (both coordination and organometallic compounds), with promising activity in TNBC (by means of cellular *in vitro*[92–124] and *in vivo* [92, 96–98,113, 115, 119, 122, 123, 127, 128] studies) have been described. The activity of coordination cationic compounds (most relevant depicted in Scheme 5) in TNBC are linked to a variety of cellular mechanisms such as DNA interactions and apoptotic cell death, among others.[93–112] Ratanaphan and co-workers focused on elucidating cellular effects on *BRCA1*-defective cells with compound **32** (**$[Ru(Clazpy)_2phen]Cl_2.8H_2O$** in Scheme 5), a Ru(II) coordinated complex with the bidentate ligand 5-chloro-2-(phenylazo)pyridine.[94] Cytotoxicity was observed in MDA-MB-231 (IC_{50} = 13.2 ± 0.3 μM, 24h) and *BRCA1*-defective HCC-1937 (IC_{50} = 1.8 ± 0.1 μM, 24h) cell lines, along with cell cycle inhibition at G2/M phase and an apoptotic mechanism of cell death. Intracellular accumulation at the nucleus, preferentially in the HCC-1937 line, was linked to a mechanism of DNA damage, which was confirmed by tumor suppressor gene *BRCA1*-related studies. Up to a 50% reduction of DNA amplification of *BRCA1* exon 11 was observed in both TNBC cell lines, with upregulated mRNA expression of p53. The use of circular dichroism and computer models determined that exposure to **32** altered the secondary structural folding of the N-terminal *BRCA1* RING protein and could act as a possible binding site. The enhanced

cytotoxicity and nuclear uptake seen in the HCC-1937 cell line suggests that the deficiency of *BRCA1* enhances the activity of **32**.[94]

A ruthenium-coordinated polypyridyl compound **33** (**RuPOP**, Scheme 5) was evaluated for cytotoxic activity in MDA-MB-231 (IC_{50} = 14.6 ± 3.1 μM, 24h) and MDA-MB-468 (IC_{50} = 78 ± 19.8 μM 24h) cell lines.[95] A transferrin receptor (TfR) competing assay revealed that pre-treatment of cells with transferrin caused a blocking effect on the internalization of **RuPOP** in a dose-dependent manner, suggesting that cellular uptake could be correlated to TfR-mediated endocytosis. Intracellular colocalization in lysosomes was observed for **RuPOP**, supporting this theory. Furthermore, a synergistic interaction between **RuPOP** and the tumor necrosis factor-related apoptosis inducing ligand (TRAIL) was discovered. TRAIL induces apoptosis in tumor cells specifically and activates multiple apoptotic pathways. Inhibition of MMP2/9 and downregulation of VEGF was also observed under RuPOP exposure.[95]

Encapsulation of ruthenium coordination complexes through liposomal or nanoparticle systems has also been explored, with the aim to increase their selectivity and activity. Shen and co-workers designed a liposome encapsulation for polypyridine compound **34 $[Ru(phen)_2dppz](ClO_4)_2$**, or **Lipo-Ru** (Scheme 5).[96] The hydrophobic nature of the resulting liposomes promotes fluorescent activity of the complex, allowing for the visualization of the **Lipo-Ru** within the cell. It was demonstrated that cellular uptake was enhanced for the encapsulated derivative, and an *in vivo* study in an orthoptic MDA-MB-231 tumor model showed major suppression of tumor growth with Lipo-Ru (a 66.9% reduction vs the control when encapsulated over a 4-week study, 5mg/kg/week).[96]

Different groups have also studied effects on TNBC zebrafish models of ruthenium coordination complexes, such as compound **35, $Ru(bpy)_2$BEDPPZ** (Scheme 5) a Ru (II) polypyridine complex coordinated with phenazine synthesized by Mei and co-workers.[97] Compound **35** was localized in the nucleus after 72h, which was linked to a mechanism of action involving DNA damage. This mechanism is associated with an increase of DNA double stranded breaks with biomarker γH2AX, and is believed to involve DNA-damage mediated apoptosis induction.[97] A toxicity screen in a transgenic Tg(fli1:EGF) zebrafish model showed suppression of MDA-MB-231 TNBC cell proliferation and metastasis with treatment.[97]

Silveira-Lacerda and co-workers reported on four cytotoxic ruthenium/phosphane mercaptopyrimidine complexes, two of which were selected for further toxicity *in vivo* testing in zebrafish models.[98] Both compounds **36a** and **36b** (Scheme 5) are cationic with two diphosphanes coordinated to the metal center, and displayed sub-micromolar cytotoxic IC_{50} values in MDA-MB-231 cells. Cell cycle arrest occurred at G_0/G_1 phase with treatment of **36a** and **36b** and an apoptotic cell death in late stages. A toxicity study in zebrafish showed no morphological or embryotoxic effects when dosed up to 100 mg L^{-1}, although hatching rate did experience a delay that was reestablished after 96h for both compounds.[98]

The enhancement of anticancer activity through possible synergistic effects by using bioactive ligands has also been explored with ruthenium coordination compounds

(all depicted in Scheme 6). The Batista group has developed a number of these complexes[99–104] which include the incorporation of ligands with known anticancer activity such as lapachol (compound **37**)[99] and cinnamic acid (compound **38**).[103] These Ru complexes have shown enhanced cytotoxic activity in MDA-MB-231 cells (**37** IC_{50} = 0.20 ± 0.01 μM, **38** IC_{50} = 1.9 ± 0.05 μM, 48h) and have shown anti-invasion and anti-migration properties and interactions with HSA in initial mechanistic studies.[99,103]

Correa and co-workers have also explored the coordination of lapachol to Ru derivatives (like compound **39 [Ru(Lap)(dppe)(bipy)]PF_6**, Scheme 6).[110] Exposure of MDA-MB-231 cells to compound **39** showed a high cytotoxic effect (IC_{50} = 0.15 ± 0.01 μM, 48h) as well as an apoptotic mechanism of cell death with an increase of sub-G1 cell cycle arrest occurring with treatment. A loss of $\Delta\Psi m$ was observed, suggesting an interaction with the mitochondrial membrane potential and indicative of early apoptosis. ROS generation was also pronounced with exposure to the complex. All this evidence taken together suggests mitochondrial involvement in the mechanism of action for **39**).[110]

Cominetti and co-workers have also focused on coordination Ru(II) compounds containing bioactive ligands.[111–113] Compound **40** [Ru(GA)$(dppe)_2$]PF_6 (**Ru(GA**) in Scheme 6) is a ruthenium(II) biphosphane complex containing a gallic acid ligand, which is a triphenol found in the plant kingdom that is active in a number of antitumor signaling pathways.[111] Compound **40** is cytotoxic in both MDA-MB-231 (IC_{50} = 0.8 ± 0.08 μM, 48h) and MDA-MB-468 (IC_{50} = 1 ± 0.01 μM, 48h) cell lines. Alterations to the cytoskeletal structure of cells were observed in a selective manner in the cancerous TNBC cell lines, suggesting early apoptotic signaling involved in the mechanism of action for **Ru(GA)**. Increased levels of pro-apoptotic factors Bax, Cas8, Cas9, and Cas3, and downregulation of anti-apoptotic factor Bcl2 confirmed an intrinsic apoptotic pathway involved. It was also determined that TfR receptors play a role in Ru(GA) transport into tumor cells. TfRs are overexpressed in cancer cells, and were silenced in cell line MDA-MB-231 through anti-TfR monoclonal antibodies s725 and s727 transfection. Cell viability was significantly altered with 53% viability seen in s725 TfR-silenced cells, compared to 27.5% in non-treated cells.[111]

An *in vivo* mouse model was also used by Cominetti and co-workers to highlight the lack of toxicity of a ruthenium-polyamine complex with bioactive ligand acylthiourea (compound **41** in Scheme 6, ***trans*-[Ru$(PPh_3)_2$(N,N-dimethyl-N′-thiophenylthioureato-k^2O,S)(bipy)]**).[113] Compound **41** is highly cytotoxic to MDA-MB-231 cells (IC_{50} = 8.81 ± 0.81 μM, 24h) with significant cell cycle arrest at sub-G1 phase and an apoptotic mechanism of cell death confirmed with qPCR and western blot analysis of pro-apoptotic genes Bax and caspase 3. An *in vivo* acute toxicity study of compound **41** was completed in swiss mice with no overall signs of behavioral distress or histopathological toxicity at a dosage of up to 50 mg/kg administered intraperitoneally over a 14-day study. However, significant DNA damage was observed during genotoxic evaluation of the mice exposed to the highest dose of the Ru complex, although this damage is considered predominately minor as only nucleoids were affected. Overall, ruthenium-coordinated complexes display a variety of cellular and mechanistic effects related to DNA damage and mitochondrial dysfunction.[113]

A number of organometallic ruthenium (II) complexes have been described as very active against TNBC cells and tumors.[105–128] Most of these compounds include a cyclopentadienyl or arene ligand (like p-cymene) and are cationic or include easily ionizable ligands like chloride (see Scheme 7). Many of the compounds display low- and sub-micromolar IC_{50} values in TNBC cell lines, and in some cases (but not all) interactions with DNA as well as interactions with proteins like BSA or HSA are described. In some other cases, effects on mitochondria have been reported. Organometallic complexes tested in murine models have particularly illuminated the potential clinical chemotherapeutic efficacy.[118–122]

Garcia and co-workers have described cationic organometallic cyclopentadienyl ruthenium complexes.[114–118] Compound **42**, termed **TM90** (Scheme 7), displayed high cytotoxicity in MDA-MB-231 cells (IC_{50} = 0.73 ± 0.12 μM, 24h), and the *in vivo* efficacy of the compound was evaluated in an orthoptic tumor model in N:NIH(S)II-nu/nu nude mice.[118] A 10-day cycle treatment with 2.5 mg/kg of **TM-90** showed around 50% decrease in tumor growth when compared to non-treated mice. **TM90** treated mice were also able to survive for up to 200 days following tumor surgical excision. Histopathological analysis showed accumulation in liver and kidney tissue, with slight accumulation in lungs, heart, and blood samples. It was extrapolated that this increase in accumulation in the kidney is due to excretion of the potential anticancer drug following treatment.[118]

Our group at Brooklyn College reported on a series of cationic organometallic ruthenium (II) complexes with varying iminophosphorane ligands like compound **43** (**Ru-IM** in Scheme 7).[119–121] Notably, **Ru-IM** is highly soluble in water (100 mg/mL) and stable as a solid exposed to ambient air. In addition, it is also stable for months in DMSO solution, and its half-life in H_2O is 2.5 days. **Ru-IM** is effective against several cisplatin-resistant cell lines but less toxic to healthy human renal proximal tubular (RPTC) cell lines. Moreover, **Ru-IM** (**43**) was found to be highly cytotoxic to the triple negative breast cancer (TNBC) cell line MDA-MB-231 (50-fold more toxic than cisplatin), with an IC_{50} value of 2.61 ± 1.2 μM (24 h). Initial mechanistic studies indicated that Ru-IM induces canonical or caspase-dependent apoptosis.[119,120] Like some other Ru compounds described here, **Ru-IM** appears to follow a mechanism of action that differs from that of cisplatin. This is because **Ru-IM**-induced cell death is not dependent on p53, and the interaction between **Ru-IM** with DNA is weak and electrostatic in nature. Additionally, **Ru-IM** was found to be highly effective *in vivo* using NOD.CB17-Prkdc SCID/J mice bearing xenograft TNBC tumors grown from MDA-MB-231cells. Specifically, a tumor reduction (shrinkage) of 56% after 28 days of treatment (14 doses of 5 mg/kg every other day) and low systemic toxicity was observed making it the most efficacious of organometallic compounds described to date in TNBC mice models. Pharmacokinetic studies showed a quick absorption of Ru-IM into blood plasma with an elimination half-life of 12.67 h, similar to those reported for other Ru derivatives in other type of cancer tumors.

Remarkably, **Ru-IM** accumulated preferentially in breast tumor tissues when compared to kidney and liver tissues, which may explain its high efficacy *in vivo* and lack of systemic toxicity.[119,120] This compound was submitted for the NCI 60 cancer cell line panel evaluation.[121] The results obtained suggest that Ru-IM is effective against 49 of

the 60 cancer cell lines tested at GI_{50} ranging from 1.7 to 3.9 μM (48-h incubation). The GI_{50} values for breast cancer cells range from 1.9 to 2.6 μM (although none of the breast cancer cell lines tested carried *BRCA1* or *BRCA2* mutations). Interestingly, the GI_{50} values for TNBC cells MDA-MB-231, colon cancer cells, and melanoma cells were below 2 μM. Recent *in vitro* data in MDA-MB-231 cells suggest that **Ru-IM** accumulates preferentially in the mitochondria, and also that possesses strong antimigratory properties.[121] Results from 2D wound healing assays and 3D transwell assays indicate that **Ru-IM** can significantly reduce migration (87.4%) and invasion (86%) of MDA-MB231 cells (values larger than those for NAMI-A, a non-cytotoxic ruthenium (III) compound depicted in Scheme 5 with antimetastatic properties *in vivo* that has been studied in different clinical trials).[121]

An *in vivo* mice study completed by Amici and co-workers demonstrated the potential of an arene Ru(II) complex containing bis(pyrazolyl)methane ligand ([Ru(p-cymene)(bis(3,5-dimethylpyrazol-1-yl)methane)Cl]Cl, **UNICAM1** (complex **44** in Scheme 7) in reducing tumor growth in TNBC cell line A17 transplanted into FVB/neuT syngeneic mice with an overall treatment of 210 mg/kg (given four times at intervals of three days).[122] While there was 57% inhibition of tumor growth observed with **UNICAM1** compared to the control, cisplatin treatment was slightly more effective (NAMI-A was also tested and a slight decrease in tumor size reported). Studies in the explanted tumor revealed an increased number of apoptotic cells and significant reduction of tumor-infiltrating regulatory T-cells. CD4+ and CD8+ T lymphocytes were upregulated following UNICAM1 treatment, while regulatory T-cell Foxp3 showed a decrease in activity. Histopathological studies show moderate kidney toxicity but low liver toxicity and revealed an increase of apoptotic cleaved caspase-3 positive cells with UNICAM1 treatment.[122]

Enhancement of the pharmacological profile of organometallic ruthenium complexes by incorporation of bioactive ligands has also proven efficacious.[123–127] Conjugation of inhibitors of DNA damage response complex poly(ADP-ribose) polymerase 1 (PARP-1) to a series of organometallic Ru(II)-arene was explored by Zhu and co-workers.[124] Compound **45** conjugated to PARP inhibitor 3-aza-5H]-phenanthridin-6-one showed moderate cytotoxicity (IC_{50} = 93.3 ± 11.4 μM, 72h) in *BCRA1* defective HCC-1937 cells and a slight increase in PARP-1 inhibition in comparison to the inhibitor alone. Compound **45** was also able to bind to DNA in a concentration-dependent manner, which was determined with the use of a luciferase-encoding plasmid DNA. Exposure to the compound negatively affected RNA metabolism, leading to transcription inhibition exhibited by bioluminescence when transfected into A2780 ovarian cancer cells with a CMV promoter and luciferase reporter gene. Transcription levels decreased 38% after 12h and 54% after 48h, suggesting repair of Ru-DNA lesions.[124]

Dyson and co-workers have reported on RAPTA-C, a Ru(II) organometallic complex that has been thoroughly studied as an anticancer agent *in vitro* and *in vivo*.[125] To enhance the anticancer activity of this complex with a focus on TNBC *BRCA1*-deficient cell lines, an analogue containing an arene-tethered ethacrynic acid ligand, (compound **46 RAPTA-EA1**, Scheme 7) was prepared and tested in *BRCA1*-deficient HCC-1937 cells.[126] This complex is cytotoxic (IC_{50} = 10 ± 0.5, 48h) with an apoptotic mechanism of cell death

and cellular accumulation in mitochondrial cell fragments as observed through ICP-MS analysis. The effects on the tumor suppressor gene *BRCA1* showed an upregulation of mRNA expression, a down regulation of protein expression, and a dose-dependent decrease of BRCA1 amplification of exon 11 with genomic DNA. *BRCA1*-competent MCF-7 cells were not as susceptible to the effects of RAPTA-EA1, suggesting that this complex may be advantageous only in *BRCA1*-defective cancers.[126]

A Ru-cyclopentadienyl family of phosphane-containing compounds with the bioactive biotin group incorporated into a chelating bipyridyl ligand was synthesized and characterized by Valente and co-workers (like **47** in Scheme 7).[127] The compounds showed biotin-avidin interaction (as confirmed by studies with a biotin quantitation kit) despite having different ancillary phosphane ligands. This suggests that the biotin transporter SMVT may be involved in the mechanistic action of these compounds. Cytotoxicity levels remained high in MDA-MB-231 cells (< 15μM for all compounds), and the compounds were evaluated in toxicity tests in zebrafish. Severe toxic effects including necrosis and cell lysis were observed for all non-biotinylated compounds, but were much less pronounced (yolk sac and pericardial sac enemas) for the ruthenium-biotin complexes.[127] The Valente group further explored the potential of bipyridine-biotin structures through the inclusion of a dibiotin ester as its R group in compound **48**.[128] This compound is cytotoxic to MDA-MB-231 cells (IC_{50} =11.6 ± 1.5μM, 24h and showed selectivity over the control MCF7 cell line. Inhibition of ATP-binding cassette (ABC) transporters was observed with the use of HEK293 cells transfected with BCRP, MRP1, and MRP2 transporters. Inhibition of multidrug resistant pump P-gp was also discovered with the use of fluorescent substrates in NIH3T3 cells, as the biotin-ruthenium complex blocked the efflux of P-gp. 3D models generated by the group showed that the addition of a long biotin-group allows interaction with more residues, which can affect P-gp transport. A 5-day acute toxicity study in zebrafish revealed that changes in intraocular distance, total body length measurement, pericardial sac size and yolk sac size at concentrations above 1.17 mg/L.[128]

While Castonguay and co-workers synthesized a number of complexes incorporating third-generation aromatase inhibitors targeted toward estrogen receptor positive breast cancers, sub-micromolar cytotoxicity was also observed in TNBC MDA-MB-231 cells (IC_{50} = 0.039 ± 0.09 μM, 48h) for compound **49**, **Ru(II)Cp**), although it did not seem to be selective when testing in the non-cancerous cell line MCF-12A.[92] A zebrafish toxicity screen showed no effect on hatching rate or mortality, which no observed abnormalities with up to a 1 μM dose of compound **49**.[92]

Ruthenium (III) and Mixed Valence Ru(III)/Ru(II) Compounds

The more inert nature of ruthenium (III) complexes makes this oxidation state less studied, although there is potential of prodrug capability. While previously mentioned ruthenium (III) compounds NAMI-A and KP1019/KP1339 (Scheme 5) have undergone phase clinical trials in other cancer types, their efficacy on TNBC is still being elucidated. Whole transcriptome analysis through RNA sequencing of NAMI-A in MDA-MB-231 cells showed selectivity to the TNBC cell line when compared to control line HBL-100 with early response genes related to direct or indirect roles in cellular invasion, metastasis, cell cycle regulation, and

cytoskeleton remodeling involved.[129] While NAMI-A displayed almost no cytotoxicity in the same TNBC cell line (IC_{50} = 840.21 ±_0.03μM, 72h) it showed tumor reduction *in vivo* (roughly 28% compared to control) in the previously mentioned study by Amici and co-workers.[122] KP1019 displayed much higher cytotoxicity in MDA-MB-231 cells (IC_{50} = 0.847 ± 0.22 μM, 24h), resistance to detachment following treatment, reduction of MMP2/MMP9 activity, and antimigratory and anti-invasion properties, although its salt analogue KP1339 did not display this activity.[130,131]

The effects of the encapsulation of Ru (III) complexes has been explored in the TNBC cell line MDA-MB-231. Santamaria and co-workers focused on the encapsulation of the ruthenium (III) compound **50a Azi-Ru (**Scheme 8), modeled after NAMI-A in nucleolipid-based nanosystems.[132–134] Particularly effective was a cationic 1,2-dioleoyl-3-trimethylammoniumpropane chloride (DOTAP) nanocarrier used in conjunction with nucleolipid complex HoThyRu, to generate an anionic **HoThyRu/DOTAP** nanosystem (compound **50b** in Scheme 8).[134] An increase in cytotoxicity was observed following encapsulation (IC_{50} = >250μM **Azi-Ru**, IC_{50} = 12.1 ± 3 **HoThyRu/DOTAP**, 48h) and autophagic cell death was observed following treatment with rapamycin and confirmed through elevated expression of autophagosome-related proteins LC3I and LC3-II. The *in vivo* efficacy of this nanosystem was also evaluated in MCF-7 xenografted athymic nude mice dosed with 15mg/kg once a week over a 28-day study, which showed significant decrease in tumor weight and volume with **HoThyRu/DOTAP** treatment and no signs of toxicity.[134]

Similarly, a solid polymer-lipid nanoparticle system (SPLN) with diruthenium (II) and (III) mixed valence complexes conjugated to NSAIDS ibuprofen and naproxen (compounds **51a/51b** in Scheme 8) by De Olivera Silva and coworkers revealed increased cytotoxicity and drug loading efficiency with encapsulation.[135] The fluorescent nature of this system showed accumulation of nanoparticles in tumor following injection of SPLNs into BALC/c mice with EMT6 breast tumor, and elimination was observed in the intestines after four hours of drug exposure.[135]

Iron Compounds

Iron (II) Compounds—Compounds containing ferrocene, a compound with a sandwich-like structure with two cyclopentadienyl rings bound to an iron (II) center, have been evaluated in TNBC cell lines.[136–155] Ferrocene and its derivatives (such as ferroquine and ferrocifen) have attractive qualities such as reversible redox properties and have displayed anticancer, antibacterial, antifungal, and antiparasitic activity.[136] Zhang and co-workers have reviewed the anticancer properties displayed by ferrocene-containing hybrids.[137] The effects on various cancer types of a broad spectrum of 103 different hybrids described over the past ten years which include but are not limited to: pyrazole, imidazole, chalcone, coumarin, indole, phenol, pyrimidine, and sugar hybrids were collected in this review.[138–155]

In terms of targeting TNBC, the review highlights ferrocene containing histone deacetylase inhibitors (HDACi) as particularly effective.[151–155] Double-stranded break repair is inhibited by these complexes as the mechanism of action interacts directly with DNA.

[151–153] The potential synergistic effects of these HDACi attached to other cellular inhibitors show promise as well.[154] Luparello and co-workers revealed the molecular efficacy of Jay Amin hydroxamic acid (JAHA), a ferrocene-containing analogue of HDACi suberoylanilide hydroxamic acid (SAHA).[153–155] SAHA (otherwise referred to as Vorinostat) is used as a treatment for cutaneous T-cell lymphoma. The incorporation of the ferrocene motif to generate the JAHA analogue (compound **52** in Scheme 8) proved effective in initial studies, with **52** displaying cytotoxicity in the MDA-MB-231 cell line (IC_{50} = 8.45 μM, 72h) along with cell cycle dysfunction at G2/M phase and increased ROS production with mitochondrial membrane dissipation, and a non-apoptotic cell death.[153] The molecular signature of both acids were evaluated with differential-display-PCR and proteomic analysis in MDA-MB-231 cells and showed that while SAHA and JAHA had similar expression levels for differentiation and growth inhibition genes gelsolin, IDI1, and VIDUP1, JAHA selectively upregulated oxidative stress related genes neurotrophic tyrosine kinase receptor type 2 (NTRK-2) and DNA repair protein RAD50.[155] This suggests that the addition of the ferrocene promotes oxidative DNA damage, which will need to be further explored.

Following publication of the review, other organometallic Fe (II) complexes containing cyclopentadienyl ligands have also been reported displaying cytotoxic effect on MDA-MB-231 cells (sub-micromolar ranges, 48h).[156]

Iron (III) Compounds—Acilan and co-workers described six coordination cationic Fe (III) complexes that were tested in MDA-MB-231 cells and showed variable cytotoxicity (IC_{50} values ranged from 6.5 to >50 μM, 24h).[157] Three of these compounds (**53a-c** in Scheme 8) showed a caspase-dependent induction of apoptosis and increase of intracellular ROS production. DNA interactions were also confirmed with COMET and DNA cleavage assays, as well as the phosphorylation of H2AX, a marker of DSBs.[157]

4. Other Metal-Based Compounds

Transition Metal-Based Compounds

Other transition metals such as rhenium, manganese, rhodium, iridium, and vanadium have also been evaluated for chemotherapeutic use with promising potential. Rhenium compounds have shown preclinical success in pancreatic, prostate, and ovarian cancers,[158,159] and similar preclinical evaluations are being done in renal cancer with iridium derivatives.[160] A recent review by Falasca and co-workers highlighted rhodium, iridium and rhenium-based anticancer agents,[161] while Inkelewicz-Stepniak and co-workers focused on vanadium compounds in another review.[162] Although these metals have not been heavily explored in the treatment of TNBC, we will highlight below the compounds that have been reported.

Rhenium and Manganese Compounds,

Many modes of action have been attributed to rhenium-based compounds which include DNA binding, phototoxicity, mitochondrial effects, enzyme inhibition, and oxidative stress regulation.[163] Mandal and co-workers investigated the cytotoxic profile of 43

organorhenium complexes, establishing IC_{50} values that ranged from sub-micromolar levels up to 6.54 μM in MDA-MB-231 cells and suggesting that rhenium-based complexes show enhanced cytotoxic properties, although not necessarily selective as similar values were observed for tested cell lines MCF7 and MCF10a.[164] Antioxidant effects were demonstrated for compound **54 Re-diSe** (Scheme 9), a rhenium (I) tricabonyl complex chelated by a diselenoether ligand synthesized by Desmaele and co-workers.[165] Compound **54** was evaluated in MDA-MB-231 cells for cytotoxic activity (IC_{50}= 48.51 ± 2.75μM, 72h), and exposure to the compound resulted in a decrease of ROS production and levels of TGF-beta1, VEGF-A and IGF-1. The di-Se ligand by itself was not as effective as the whole complex, suggesting that the addition of rhenium is advantageous.[165] Manganese-based compounds have had recent preclinical success in nanoparticle-based cancer therapy,[166] although the efficacy of manganese complexes in TNBC has yet to be fully elucidated. Binti Salahen and co-workers analyzed compound **55** (Scheme 9), a beta-diiminato manganese^{III} complex coordinated to an indole Schiff base, which showed a caspase-dependent apoptotic cell death in MDA-MB-231 cells.[167] An *in vivo* acute oral toxicity test with doses up to 300 mg/kg/day was also completed on Sprague Dawley rats by this group, with results showing negligible kidney and liver toxicity. Combination treatments with doxorubicin and tamoxifen were also evaluated, showing synergistic and antagonistic effects, respectively.[167]

Rhodium and Iridium Compounds

Some rhodium (III) complexes have also been evaluated as potential TNBC agents.[168–170] Leung and co-workers synthesized cyclometallated Rh(III) compound **56** (Scheme 8) which also acts as a lysine-specific demethylase 5A (KMD5A) inhibitor.[170] KMD5a is a histone demethylase that promotes tumorigenesis and metastasis. The phenanthroline ligand enhanced inhibitory activity in a structure activity relationship and an *in vivo* study in 4T1 xenografted BALB/c mice treated with **56** showed an inhibition of tumor growth up to 48% at 4mg/kg over 9 days. However, cisplatin and doxorubicin treatments showed increased inhibition, albeit with severe toxic effects to spleen and kidney organs.[170]

The efficacy and mechanism of iridium compounds in TNBC has not yet been elucidated. Two cyclometallated Ir (III) complexes by the previously mentioned Leung group did not display anticancer activity in TNBC cells, although the rhodium-based compounds from the same study did.[171] A preliminary study with two organo-iridium (I) complexes synthesized by Sadler and co-workers showed enhanced anti-proliferative properties in TNBC cell lines MDA-MB-468 and OCUB-M in a National Cancer Institute (NCI) 60 cell line screen, in particular, the iridium-phenylazopyidine complex (**57** in Scheme 9).[171]

Vanadium Compounds

Compounds containing the transition metal vanadium enhance redox reactions and interactions with nucleic acids, which promote mechanisms of cell death such as cell cycle arrest and DNA damage in various cancer types.[172,173] Recent studies in MDA-MB-231 cells have shown apoptotic and autophagic cell death with notch pathway inhibition of a vanadium-based complex, compound **58**, a oxidovanadium (IV) complex by Gonzalez-Baró and co-workers with a chelating ligand made from the condensation reaction of

o-vanillin and 2-thiophenemethylamine.[174] Cellular studies in MDA-MB-231 cells showed cytotoxicity (IC_{50} = 29 ± 1.7 μM, 48h), an apoptotic cell death, antimigratory activity, and increased ROS production.[174]

Main Element-Based Compounds

Tin and Germanium Compounds—Brtko and co-workers studied a series of eight triphenyltin, tributyltin, and tributylgermanium organometallic compounds for anticancer activity.[175] Most of these compounds showed poor cytotoxic effects in MDA-MB-231 cells (ranging from roughly 70 – 600 μM, 72h). Germanium containing derivatives, in particular, displayed limited inhibition, but were also found to reduce migration in a wound healing assay. Further studies revealed that tributyltin derivatives had a caspase-3/7 dependent apoptotic cell death.[175]

5. Highlighting Promising Agents Studied in TNBC Mammal Models

The data that we have collected in this review points to the extraordinary potential that a number of metal-based compounds hold as chemotherapeutic agents for TNBC. As indicated by authors of other reviews on metal-based drugs, it is extremely difficult to establish accurate SARs due to the large variety of metals/oxidation states/ligands studied, the heterogeneity of TNBC cell lines and *in vivo* models used, and the very diverse biological assays, targets evaluated and methods employed. We found that most of the compounds reported displayed IC_{50} values in the low micromolar to sub-micromolar range, with a few compounds selective when compared to non-cancerous cell lines. For most metals, apoptotic behavior was described and for platinum and mostly ruthenium compounds, antimigratory activity was described (indicating a potential antimetastatic behavior). We found a handful of examples for most metals where the evaluation *in vivo* indicated lack of systemic or general toxicity. Mechanistic studies show a diverse and at times selective and multi-modal action, with targets that include DNA, mitochondria, and proteasome, as well as the inhibition of cancer related cell signaling pathways. For a number of compounds, *in vivo* studies zebra fish models and mammals (mice and rats) were performed (either to evaluate the toxicity or for efficacy studies) but for several of these compounds the mice models evaluated were not for TNBC. We have found that for most of the reports, stability and solubility studies of the compounds are missing. In this section we list and highlight the compounds for which its efficacy in TNBC mammal (mice) models has been studied until September of 2020 (Table 1 and Figure 4). The twelve compounds were already described in previous sections. All of these complexes show the capability of tumor growth inhibition ≥ 46%, and a Ru compound reported by our laboratory (compound **43**, **Ru-IM**) shows a remarkable 56% average tumor shrinkage for all animals.[119,120] These compounds (with exception of Ru compound **44**[122]) show little or no systemic toxicity as determined by assessment of body weight loss and animal behavior, acute toxicity or histopathology studies. Moreover, detailed pharmacokinetic studies for compound **43** indicated a preferential accumulation of this compound in the breast tumor.[119,120]

For these compounds, preliminary mechanistic studies in TNBC cell lines and tumors were also performed. For platinum (II) and (IV) compounds, one gold (III), and a rhodium

(III) derivative, direct DNA damage is seen through DNA platination and direct injury (Pt compounds **5**, **11**, **12**),[31,38–40] upregulation of γ-H2AX activity (Pt compounds **12**, **13**, Ru compound **34**)[39,40,96] as well as inhibition of HDAC activity (Au compound **27**,[63] Rh compound **56**[170]). Inhibition of proteasomal activity has been demonstrated for gold (III) dithiocarbamate compounds (**25**, **26**)[61,62,73] and shows a tie to PARP cleavage.[63,66,67] Mitochondrial functions are also targeted by certain compounds, leading to the induction of pro-apoptotic factors and caspase dependency (Ru compounds **34**, **43** and **44**)[96,119,120, 122] as well as increased ROS production (Pt compound **12**).[39] Anti-inflammatory action is reported through the reduction of COX2 and related proteins (Pt compound **13**[40] and Ru compound **44**[122]) and cellular pathways such as p27 (Au compounds **25** and **26**,[61,62,73] Rh compound **56**[170]) are implicated as possible targets of anticancer activity.

Due to the severity of prognosis and survival, the intracellular mechanisms involved in TNBC progression are being heavily investigated. This knowledge has led to the development of a number of drugs currently in clinical trials, with specific cellular targets involved in their mechanism of action that show similarities to the metal-based drugs we have reviewed. A number of PARP inhibitors are being evaluated with varying but promising results,[176] and HDAC inhibitors are also being tested due to their direct interactions with DNA.[177] Interestingly, a recent study has shown a greater reliance of TNBC cells on glycolysis and mitochondrial metabolism when compared to ER+ cells, emphasizing the role of mitochondrial targeting drugs in potential TNBC treatment.[178] Thus, the *in vivo* activity of the metal compounds we describe above paired with the clinical investigation of other anticancer drugs targeting the same or similar mechanisms, suggests that these compounds have potential to aid in the treatment of TNBC. With further evaluation to show a more complete mechanistic profile of the aforementioned drugs, there is a possibility of future clinical success.

6. Conclusions and Outlook

In conclusion, we have demonstrated that metal-based compounds hold enormous potential as chemotherapeutics in the treatment of TNBC either alone or in combination therapy. We found that a large variety of metal-based compounds containing different metals/oxidation states and ligands have resulted efficacious *in vitro* and *in vivo* TNBC models. These compounds have diverse modes of action and mechanisms that differ from those of cisplatin and other FDA-approved platinum drugs, and that coincide in specific cases with TNBC targets explored in clinical trials. This expands the current perception that only classical DNA-intercalating platinum compounds can be of use for TNBC treatment (specifically for patients with *BRCA*-mutations). In general, a multi-faceted approach that targets pathways associated with TNBC, regardless of *BRCA* mutation status, is crucial for treating all TNBC patients.

Importantly, while the use of bioactive ligands for a rational design of anticancer agents has afforded derivatives with improved biological activity/pharmacological profiles, a relevant number of compounds not containing such ligands (mostly of ruthenium and gold) have resulted highly efficacious with almost no systemic toxicity *in vivo*. Advanced mechanistic studies (especially *in vivo*) will be necessary to further translate the potential of the metal-

based compounds to the clinic. Moving forward, researchers will have to pay attention not only to mechanistic data, but also to the stability of the compounds in human plasma before proceeding with more advanced pharmacological studies. In addition, most of the compounds explored (with the exception of Pt compound **2**) are not good candidates for oral administration. Research to improve the solubility in water and better adsorption of any potential TNBC agent is thus needed. In this review, we have briefly highlighted that the encapsulation of some of these compounds in liposomes or their self-assembly into nanoparticle carriers, can improve their pharmacological profile. Recent advances in nanotechnology and targeted therapies can certainly help develop improved metal-based TNBC agents with translational potential.

Lastly and as for most cancer and disease research, the large majority of the TNBC cells employed are those derived from patients of white ethnicity (MDA-MB 231, *BCRA1* defective HCC-1937, and *BCRA1* gene mutation carrier L56Br-C1). Only ca. 5% of *in vitro* studies performed have been based on cells of patients of black ethnicity (such as MDA-MB 468, MB157, or BT-20). To the best of our knowledge, no other TNBC cells from patients from different ethnicities have been tested with metal-based compounds. Moreover, all *in vivo* mice models tested are either murine (4T or 4T1) or derived from MDA-MB 231 xenografts. Overall, further understanding of the biological basis of responsive to treatment for TNBC is needed that considers racial differences, and we anticipate advances in this area with metal-based compounds.

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This review is dedicated to all scientists affected by breast cancer whom we want to strongly support.

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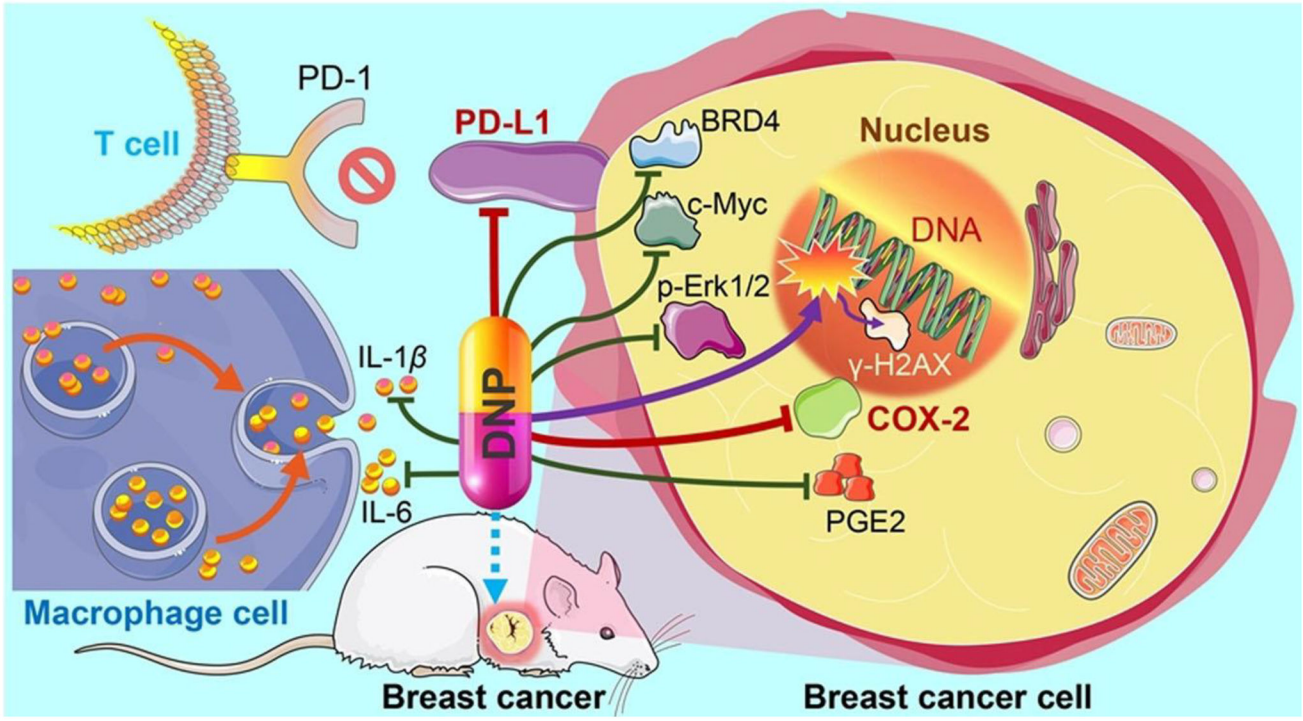


Figure 1.
Proposed anticancer mechanism of action for compound **13** (DNP). Reproduced with permission from Ref. [40]. Copyright 2020, Wiley-VCH.

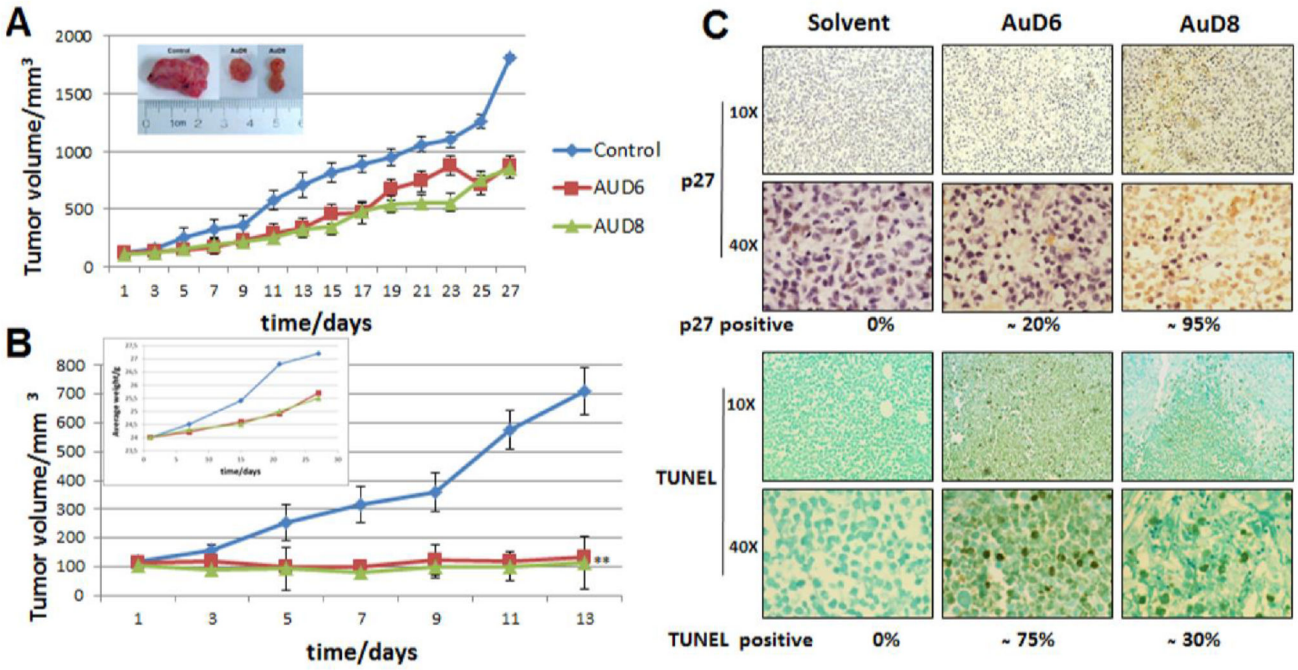


Figure 2.

Antitumor activity *in vivo* on MDA-MB-231 xenografts of gold(III)-dithiocarbamato peptidomimetics. Female nude mice bearing MDA-MB-231 tumors were treated with either vehicle (control) or the compounds **26a** (**AuD6**) and **26b** (**AuD8**) at 1 mg kg^{-1} d^{-1}. A, Inhibition of xenograft growth by both complexes. Tumor volumes were measured every other day using a caliper. Points represent the mean ± SD (bars) of seven mice per group. The insert depicts representative tumors from each treatment group; * = $p<0.05$. B, if only the most responsive mice are considered, the xenograft growth inhibition is greater. The insert shows average weights of mice over time; ** = $p<0.01$. C, Immunohistochemical p27 and TUNEL staining of tumor samples indicates proteasome inhibition and apoptosis as a result of both compounds. Stronger p27 staining is observed following **26b** (**AuD8**) treatment, and more TUNEL positive cells are observed following **26a** (**AuD6**) treatment. Brown colored cells are considered positive. Reproduced with permission from Ref. [73]. Copyright 2014, PLOS journals.

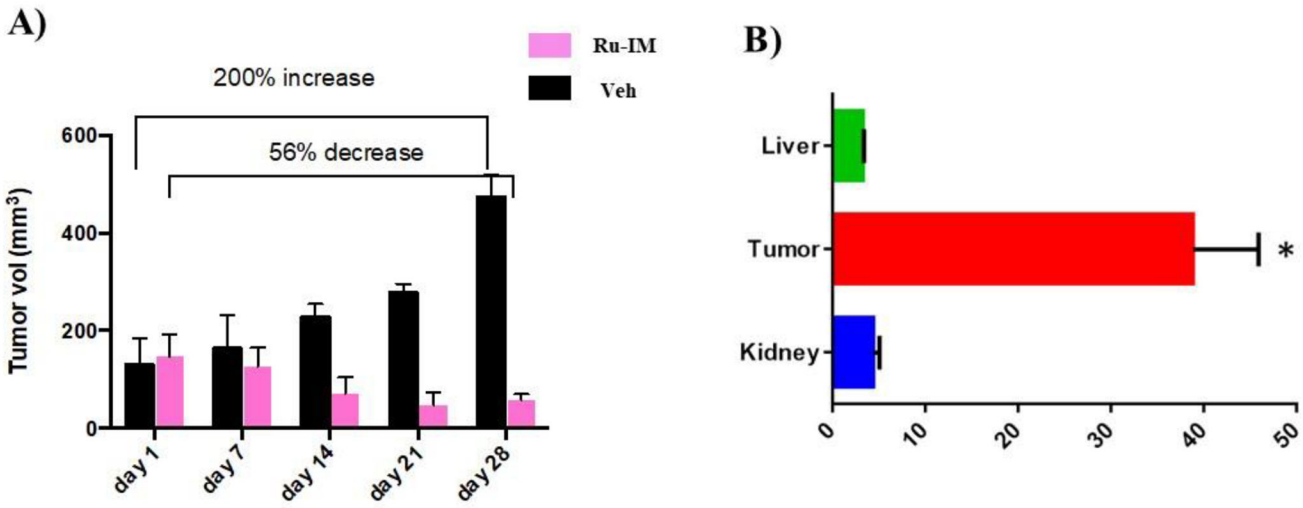


Figure 3.

A) % of reduction of tumor burden in a cohort of 12 female NOD.CB17-Prkdc scid/J mice inoculated subcutaneously with 5×10^6 MDA-MB-231 cells. The treatment started when tumors were palpable (5–6 mm diameter). 6 mice were treated with compound **43** (**Ru-IM**) (pink bars), 6 were treated with the vehicle 100 μl Normal Saline (0.9% NaCl) (black bars). **43** was administered in the amount of 5 mg/kg/every other day. B) Compound **43** (**Ru-IM**) ruthenium content in tissues at the end of efficacy study. Data represents mean ± SD. N = 3; * indicates $P < 0.05$. Reproduced with permission from Ref. [119]. Copyright 2014, ACS journals.

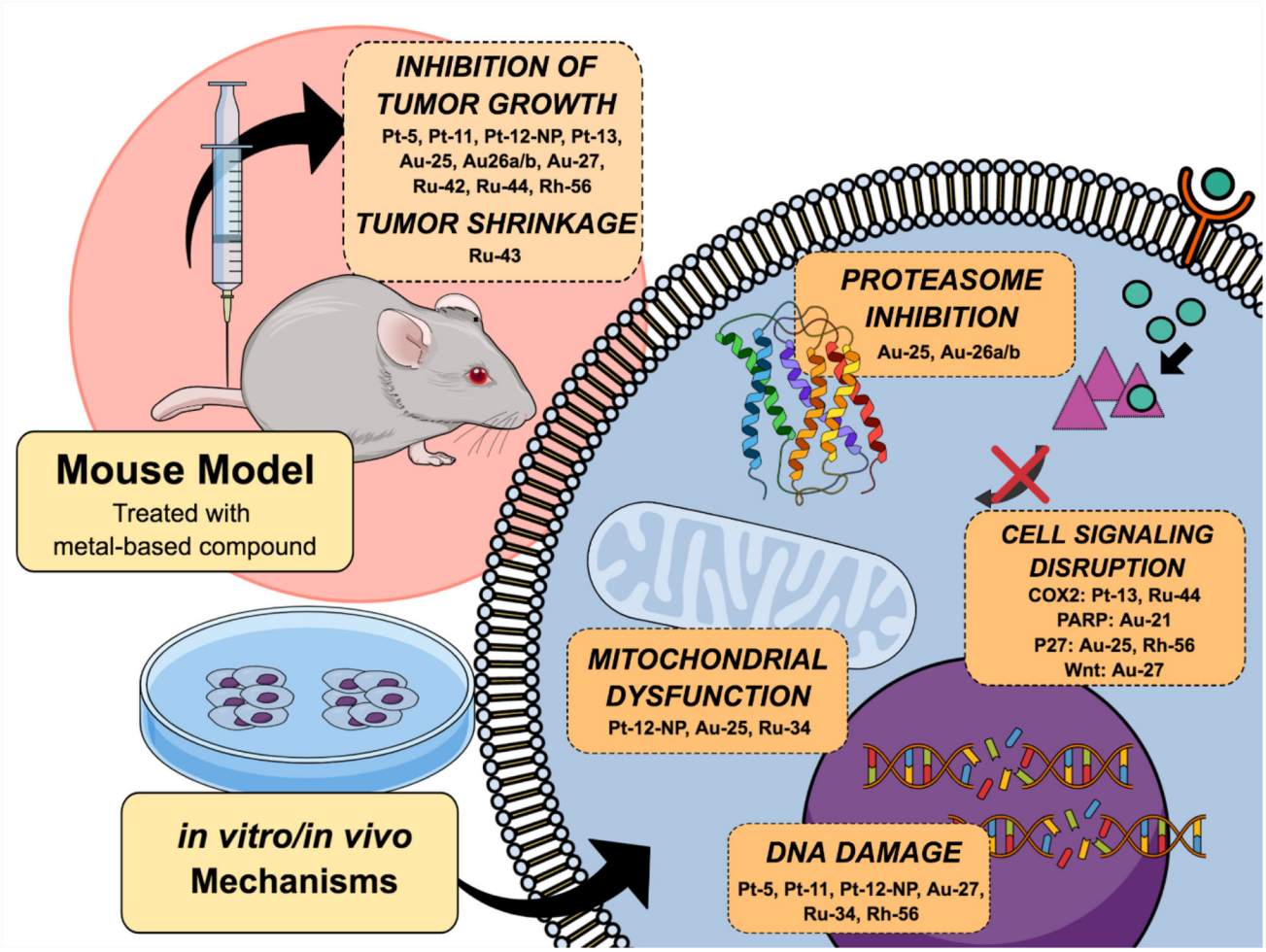
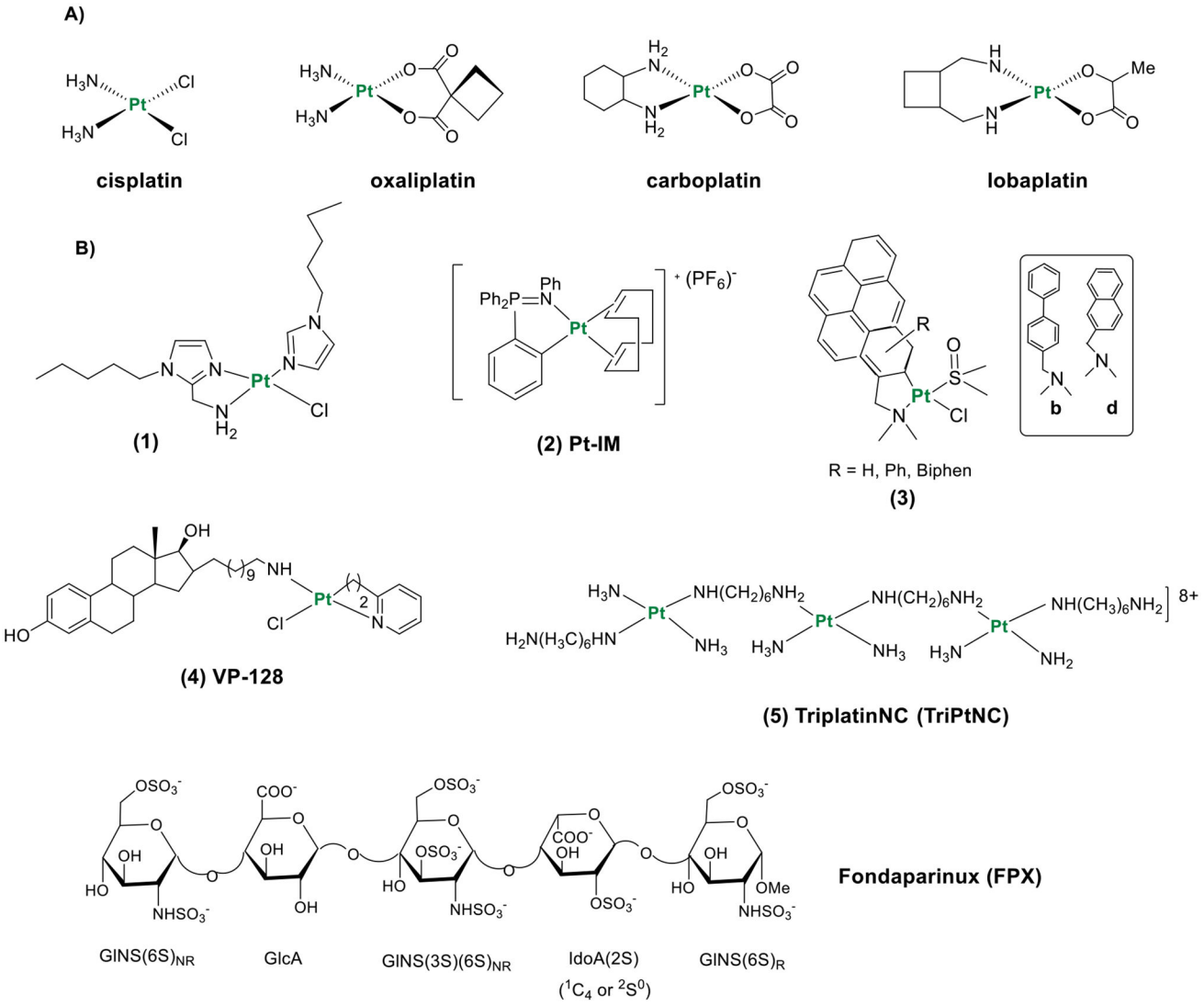


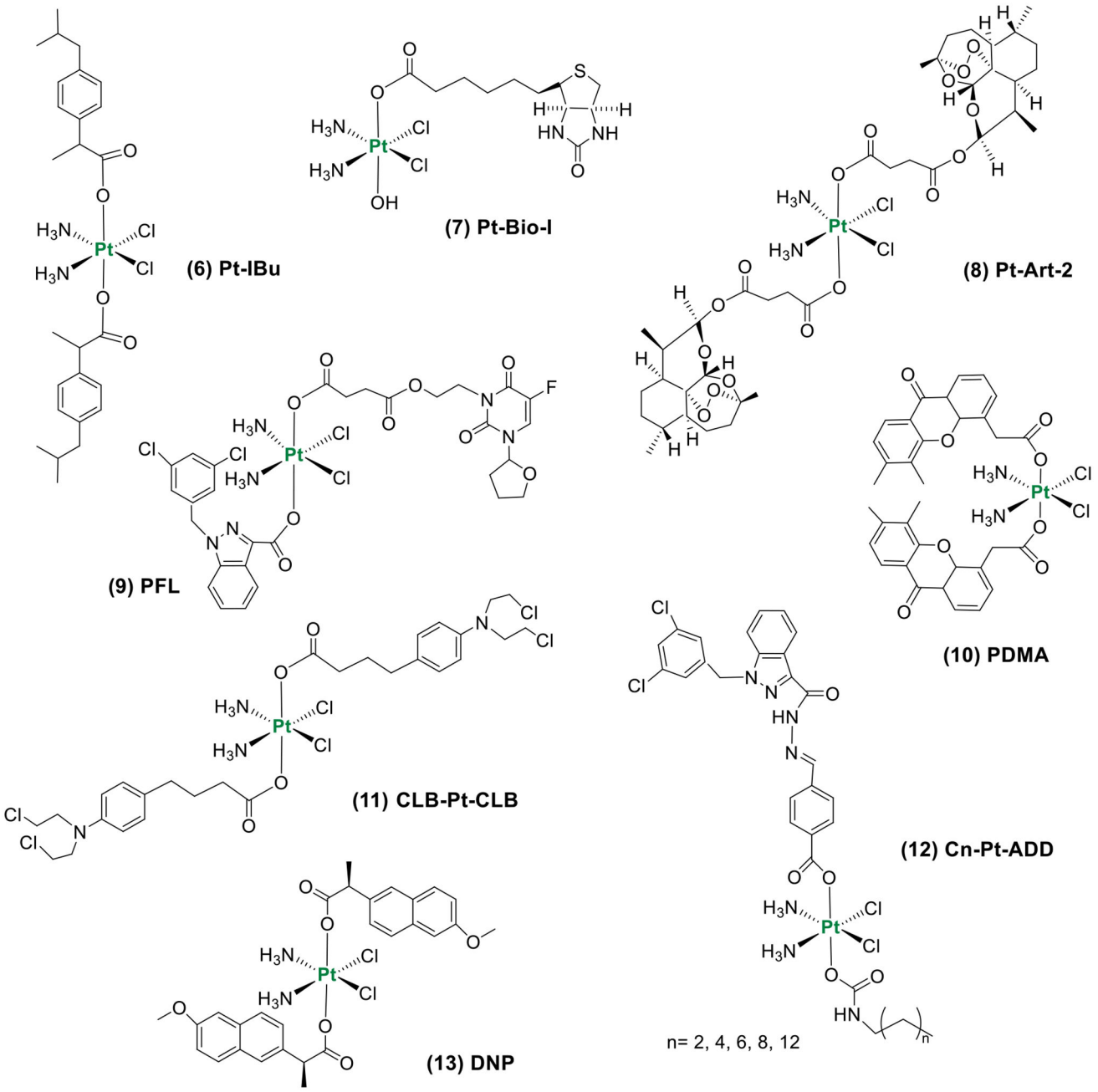
Figure 4.

Schematic of the metal-based compounds explored in TNBC mice models and their major mode of actions. Compounds are indicated by the metal contained and by the number provided to these compounds through the text.

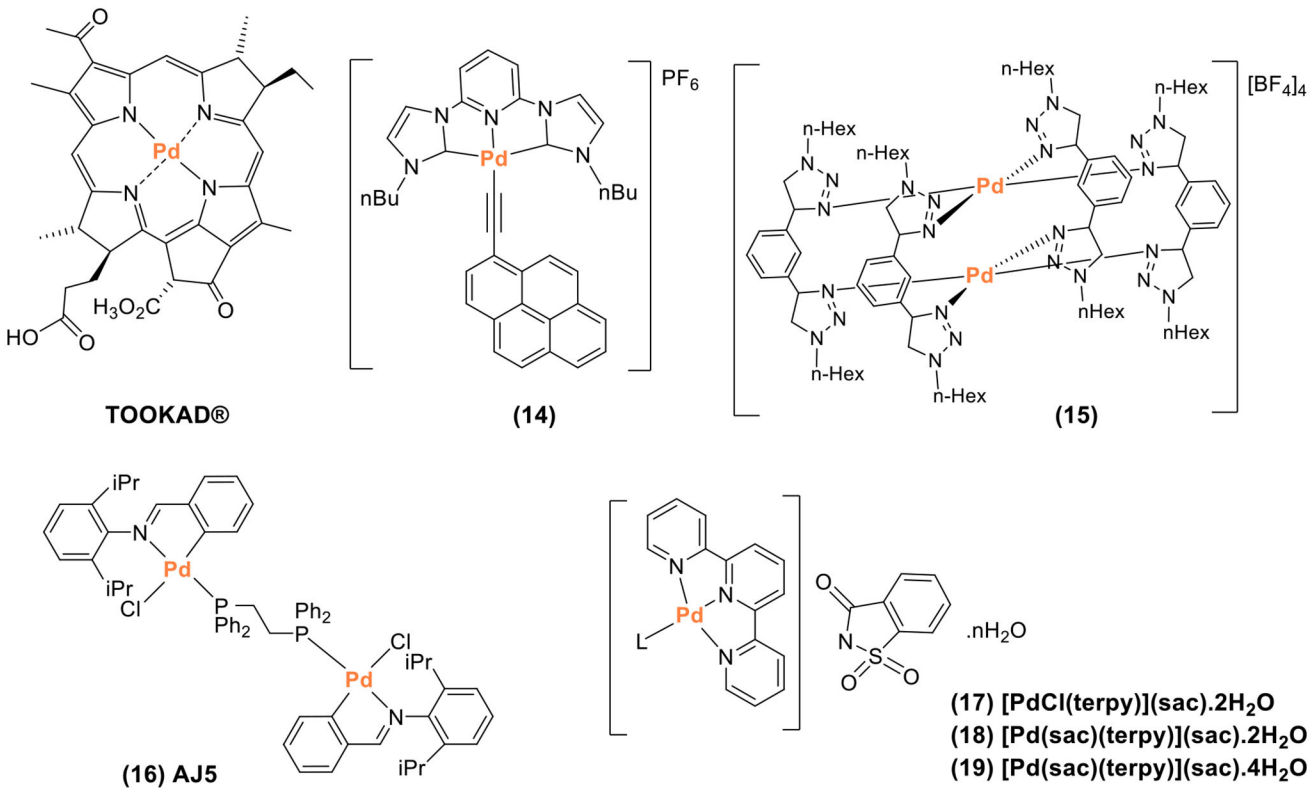


Scheme 1.

A) Platinum (II) compounds used in TNBC clinical trials (cisplatin, oxaliplatin, carboplatin, and lobaplatin)[8,17–22] or, **B)** in selected preclinical studies (**1–5**).[23–31]

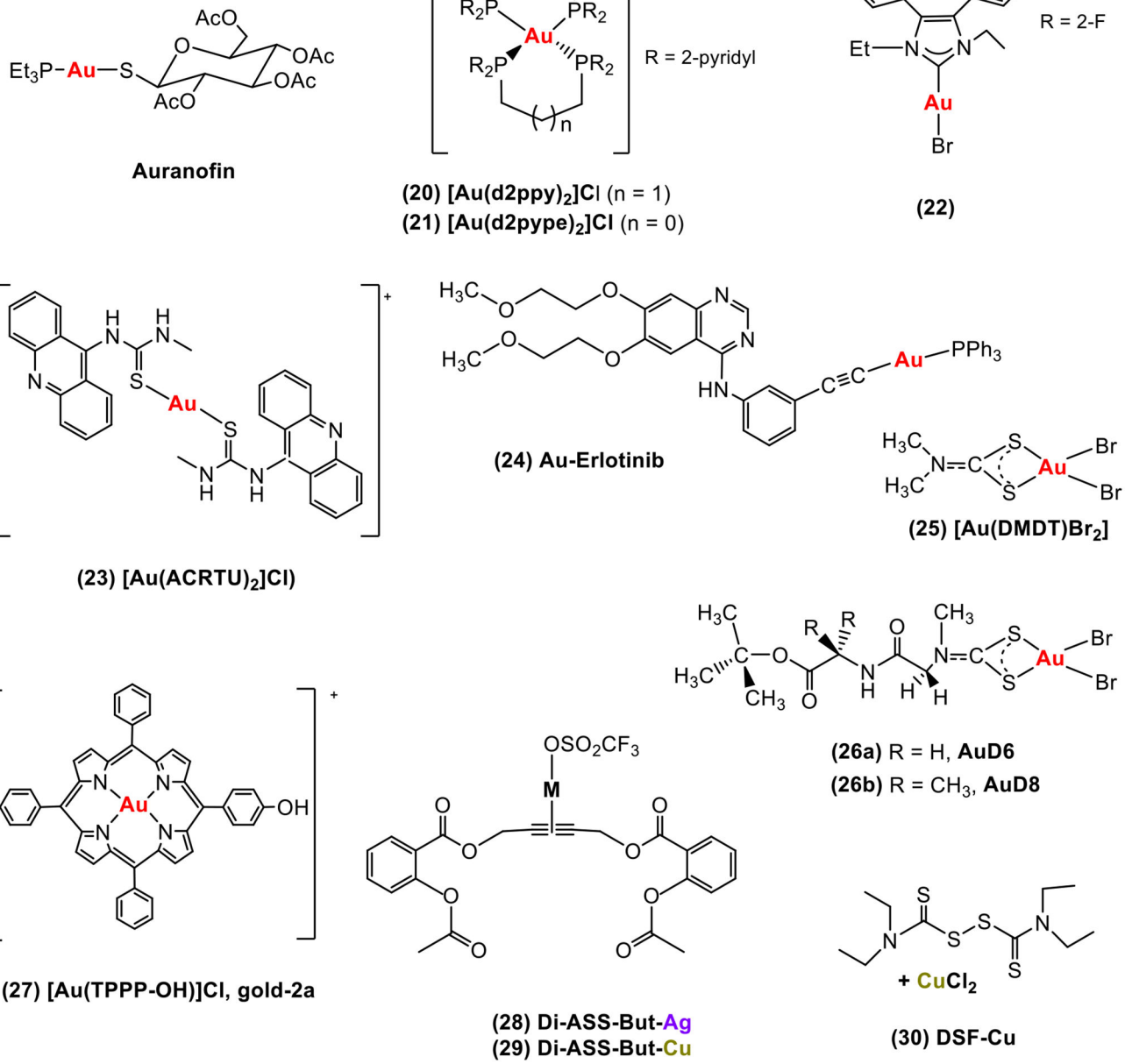


Scheme 2.
Selected platinum (IV) compounds used in preclinical TNBC studies.[33–40]



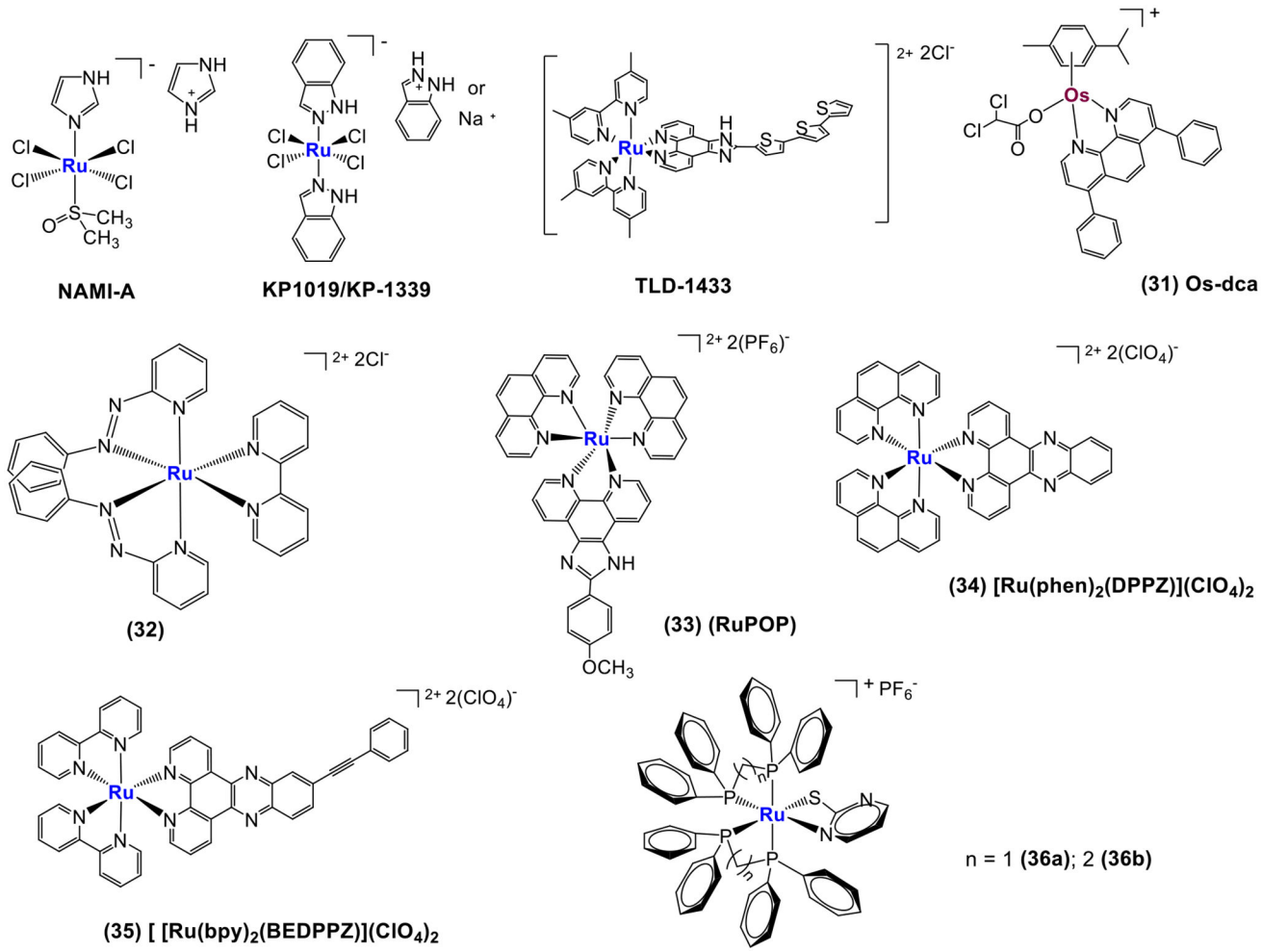
Scheme 3.

A palladium (II) used in the clinic for prostate cancer (TOOKAD®) and some selected palladium (II) compounds that have undergone preclinical TNBC studies.[43–53]



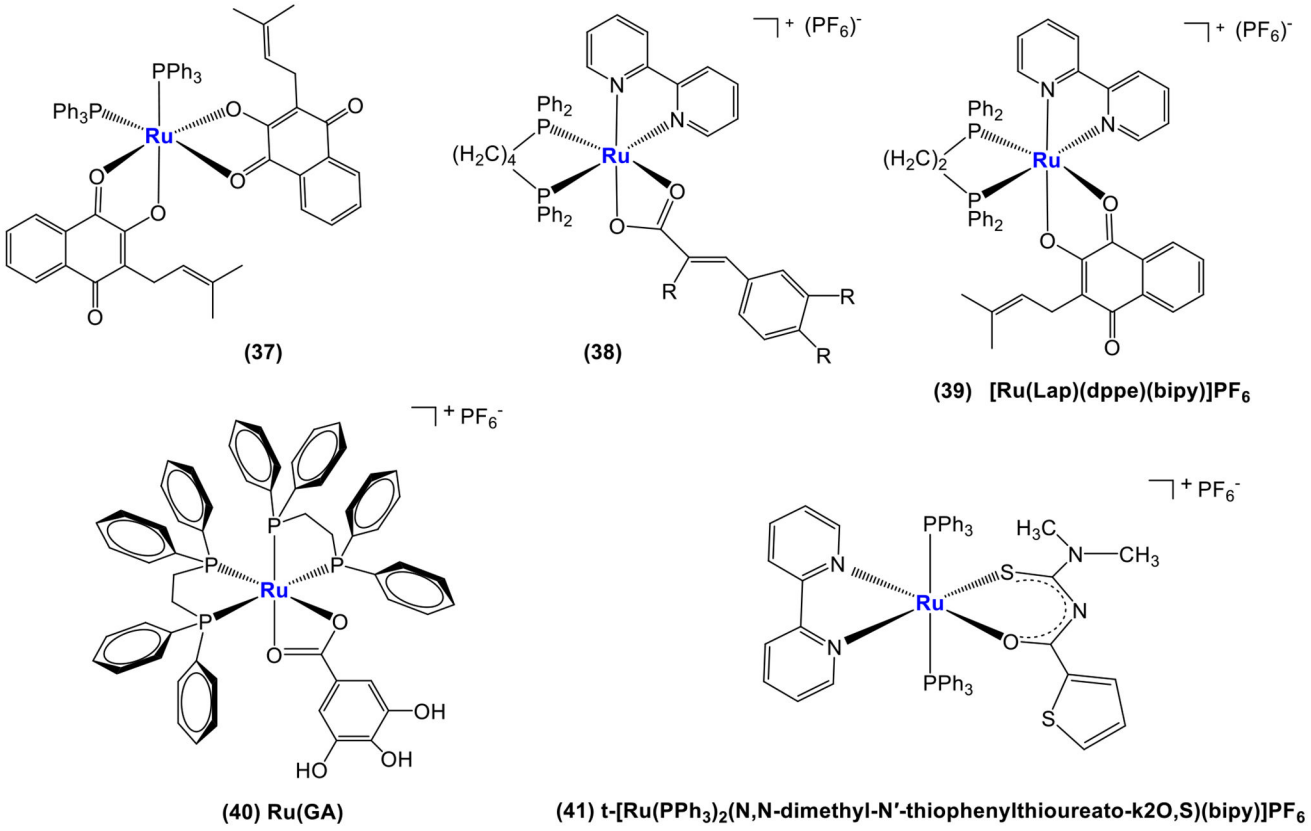
Scheme 4.

Auranofin, a gold(I) compound currently being studied in clinical trials for non-small cell lung and ovarian cancer,[54] and some selected gold, silver and copper compounds that have undergone preclinical TNBC studies.[55–74]



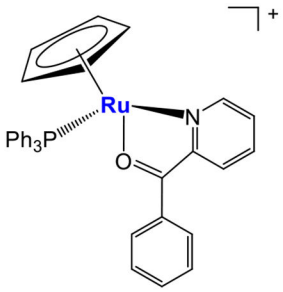
Scheme 5.

Three ruthenium (III derivatives) that underwent clinical trials for colorectal cancer (NAMI-A, KP1019/KP1339), a Ru(II)-based photosensitizer (TLD-1433) with FDA fast track designation in phase II clinical trials for treating non-muscle invasive bladder cancer, and some selected osmium (II) and ruthenium (II) coordination compounds that have undergone preclinical TNBC studies.[89, 94–98]

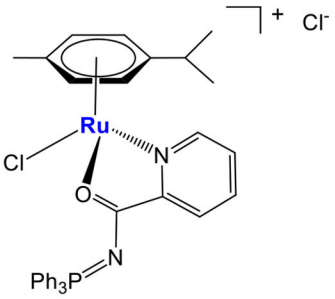


Scheme 6.

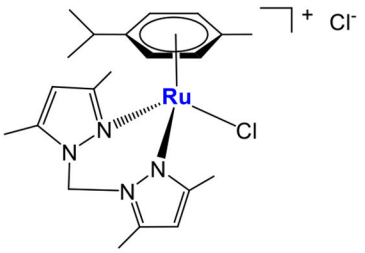
Ruthenium (II) coordination compounds containing biologically active ligands that have undergone preclinical TNBC studies.[99, 103, 110, 111, 113]



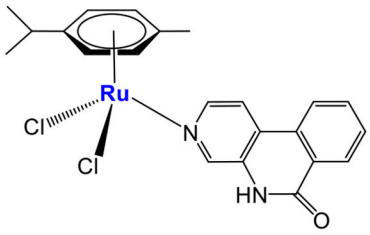
(42) TM-90



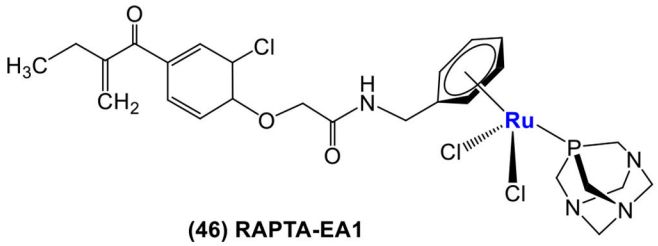
(43) Ru-IM



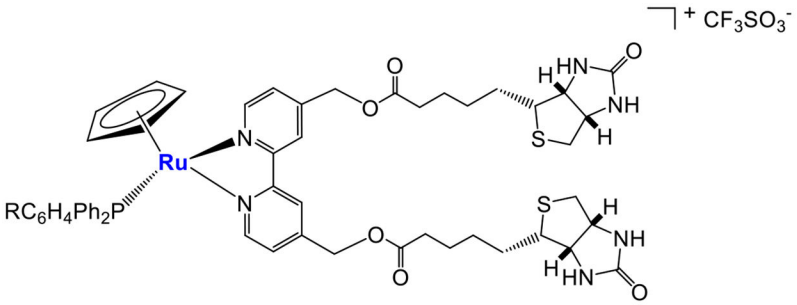
(44) UNICAM-1



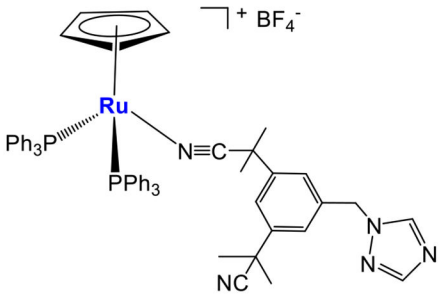
(45)



(46) RAPTA-EA1

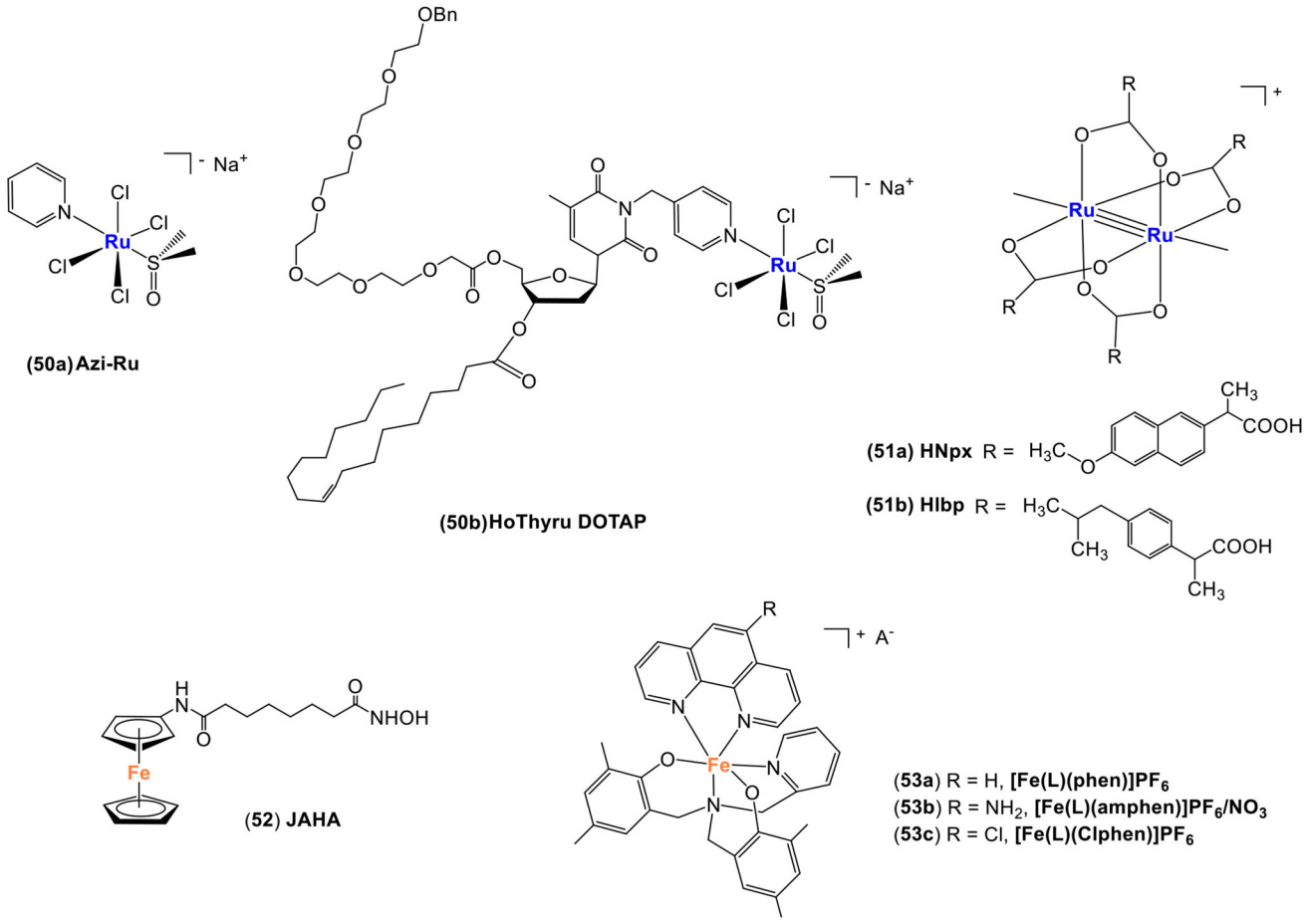


(47) R = H
(48) R = OCH3



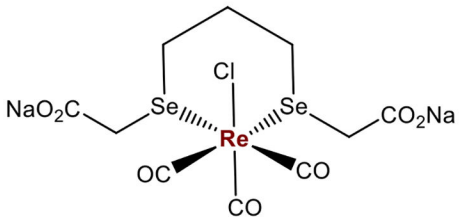
(49) Ru(II)Cp

Scheme 7.
Ruthenium (II) organometallic that have undergone preclinical TNBC studies.[92, 118–128]

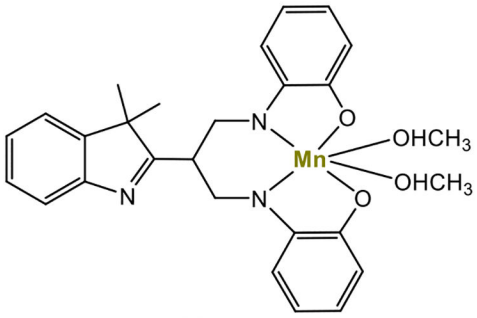


Scheme 8.

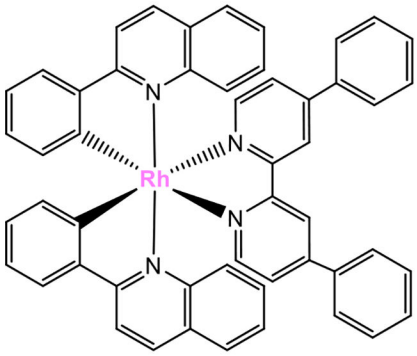
Ruthenium (III), Ru(III)/Ru(II) mixed valence, Fe(II) and Fe(III) compounds that have undergone preclinical TNBC studies.[134–136, 153–155, 157]



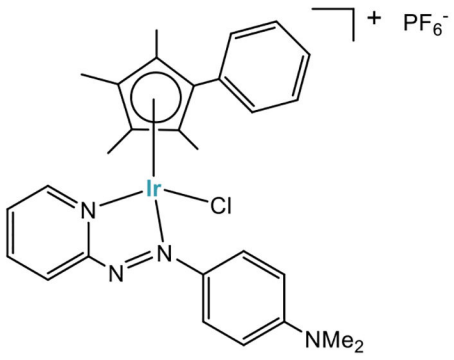
(**54**) **Re-diSE**



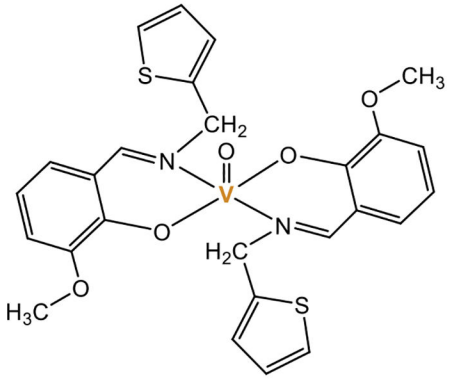
(**55**)



(**56**) **KDM5A-inhibitor**



(**57**)



(**58**)

Scheme 9.

Miscellaneous metal-based compounds that have undergone preclinical studies in TNBC cells.[165, 167, 170, 171, 174]

Table 1.

Summary of the in vitro/in vivo activity and preliminary mechanisms of all metal-based compounds tested in TNBC mice models.

Metal Compound Name (Chart)	No	In vitro & In vivo Efficacy Studies Selectivity, Cell death, Migration, Angiogenesis, Toxicity, & PK analysis			Mechanistic Studies		Ref
		***In vitro* antiproliferative activity/cell lines/selectivity/ antimigration/ antiangiogenic effects**	***In vivo* efficacy (model)**	**Toxicity & PK analysis**	***In vitro***	***In vivo***	
Pt Pt (II)							
TriPtNC (Chart 1.B.)	**5**	MDA-MB-231	Reduction of lung metastases and tumor load (female immune intact BALB/c mice with 4T1-luc2 murine breast cancer cells)	N/A	Cytotoxicity through DNA targeting. Antimetastatic action through heparan sulfate (HS) interference (inhibition of HS cleavage)	N/A	31
Pt (IV)							
CLB-Pt-CLB (Chart 2)	**11**	MDA-MB-231 Migration prevented (59%)	Inhibition of tumor growth (59%) (MDA-MB-231 xenograft Balc/C nude mice)	No observable toxicity (histo-pathology study)	Induction of apoptosis via DNA platination/ fragmentation upon reduction by ascorbic acid or GSH	N/A	38
Pt-ADD-PGE (Chart 2)	**12-NP**	MDA-MB-231 Apoptosis	Inhibition of tumor growth (59%) (MDA-MB-231 xenograft Balc/C nude mice)	No observable toxicity (acute toxicity & histo-pathology studies)	Direct DNA injury and mitochondria associated apoptosis	Reduction of Ki67 expression, increase of caspase-3 cleavage and γ-H2AX expression	39
DNP (Chart 2)	**13**	MDA-MB-231 Migration prevented (66%)	Inhibition of tumor growth (46%) (MDA-MB-231 xenograft Balc/C nude mice)	No observable toxicity (acute toxicity study)	Downregulation of expression of COX-2 and PD-L1. Inhibition of secretion of prostaglandin. Reduction of the expression of BRD4 and phosphorylation of Erk1/2. Oncogene c-Myc blockage. DNA intercalation and upregulation of γ-H2AX.	Downregulation of expression of COX-2 and PD-L1	40
Au Au(III)							
[Au(DMDT)Br_2] (Chart 4)	**25**	MDA-MB-231 Apoptosis	Inhibition of tumor growth (59%) (MDA-MB-231 xenograft female athymic nude mice)	Little systemic toxicity observed (body weight & behavior)	Inhibition of 26S proteasome (accumulation of ubiquinated proteins and proteasome target p27, and apoptosis induction)	Proteasomal inhibition and apoptosis induction	61 62

Metal Compound Name (Chart)	No	In vitro & In vivo Efficacy Studies Selectivity, Cell death, Migration, Angiogenesis, Toxicity, & PK analysis			Mechanistic Studies		Ref
		***In vitro* antiproliferative activity/cell lines/selectivity/ antimigration/ antiangiogenic effects**	***In vivo* efficacy (model)**	**Toxicity & PK analysis**	***In vitro***	***In vivo***	
AuD6 **AuD8** (Chart 4)	**26a** **26b**	MDA-MB-231	Inhibition of tumor growth (53% - 85%) (MDA-MB-231 xenograft athymic nude mice)	Little systemic toxicity observed (body weight & behavior)	Inhibition of 26S proteasome (accumulation of ubiquinated proteins and proteasome targets p27, IkB-a, and apoptosis induction)	Proteasomal inhibition and apoptosis induction	73
[Au(TPPP-OH)]Cl Gold-2a (Chart 4)	**27**	MDA-MB-231 Apoptosis	Inhibition of tumor growth & complete tumor remission with 2 bolus treatment (50% animals) (MDA-MB-231 xenograft athymic nude mice)	Little systemic toxicity observed (body weight & behavior)	Inactivation of Wnt/b-catenin signaling through transcriptional regulation. HDAC inhibition (regulates HA at the promoter regions of genes involved in Wnt/b-catenin signaling)	N/A	63
Ru Ru(II)							
Lipo- Ru (Chart 5)	**34**	MDA-MB-231 Apoptosis	Inhibition of tumor growth (66.9%) (MDA-MB-231 orthoptic athymic nude mice)	No apparent morphological changes (histo-pathology study) No observable toxicity (acute toxicity study)	Increased γ-H2AX expression, caspase activity, and PARP cleavage	Increased expression of intrinsic and extrinsic apoptotic proteins	96
TM90 (Chart 7)	**42**	MDA-MB-231 Necrosis	Inhibition of tumor growth (>50%) (MDA-MB-231 orthoptic N:$NH_{(S)}$II-*nu/nu* mice)	Accumulation in liver and kidney tissue (histo-pathology study)	N/A	N/A	118
Ru-IM (Chart 7)	**43**	MDA-MB-231 Selective (RPTC Cells) Apoptosis Migration/Invasion prevented (87%/86%)	Tumor shrinkage (56%) (MDA-MB-231 xenograft NOD.CB17-Prkdc SCID/J mice)	No observable toxicity (acute toxicity study). Detailed PK analysis (preferential accumulation tumor)	Upregulation of pro-apoptotic factors Noxa, Puma, and Bim, pP53 independent and caspase dependent mechanism.	N/A	119 120 121
UNICAM1 (Chart 7)	**44**	MDA-MB-231, A17 Apoptosis	Inhibition of tumor growth (~50%) (A17 xenograft FVB syngeneic mice)	Little systemic toxicity observed (histo-pathology study)	Increased expression of caspase-3	Inhibition of angiogenesis, increase of apoptotic cell death, Reduction of COX2 and Foxp3 levels and increase of tumor infiltrating regulatory T-cells CD4+ CD8+	122

Metal Compound Name (Chart)	No	In vitro & In vivo Efficacy Studies Selectivity, Cell death, Migration, Angiogenesis, Toxicity, & PK analysis			Mechanistic Studies		Ref
		In vitro antiproliferative activity/cell lines/selectivity/ antimigration/ antiangiogenic effects	*In vivo* efficacy (model)	Toxicity & PK analysis	*In vitro*	*In vivo*	
KMD5A-inhibitor (Chart 9)	**56**	MDA-MB-231, 4T1	Inhibition of tumor growth (>48%), (4T1 xenograft BALB/c mice)	Little systemic toxicity observed (acute toxicity study)	KMD5A and H3K4me3/2inhibitor, p27 upregulation	Elevated levels of H3K4me2/3 and p27	170