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Review

A critical review of palladium organometallic anticancer agents

Thomas Scattolin,[1,2] Vladislav A. Voloshkin,[1] Fabiano Visentin,[2,*] and Steven P. Nolan[1,*]

SUMMARY

With the aim of overcoming the well-known limitations of platinum-based antineoplastic drugs, recent efforts have focused on the development of new anticancer agents containing metals other than platinum. Among these agents, organopalladium compounds have received significant recent attention due to their generally high stability under physiological conditions. A significant number of these compounds have shown promising *in vitro* and *in vivo* antiproliferative activity toward several cisplatin-sensitive and cisplatin-resistant tumors and have sometimes exhibited a different mechanism of action compared to platinum-based drugs. In this review, recent advances in the field of organopalladium compounds as potential anticancer agents are discussed.

INTRODUCTION

Cancer represents a widespread and heterogeneous class of pathologies that every year cause ~10 million deaths worldwide, in spite of the different therapeutic approaches currently available.[1] Approximately half of the cancer patients are treated with chemoradiotherapy, and most of the protocols include the use of platinum-based antineoplastic agents.[2] The severe limitations of cisplatin and its second- and third-generation derivatives, which are ascribable to non-negligible side effects on liver, kidneys, and brain and intrinsic or acquired resistance phenomena observed in some types of tumor,[3] have prompted the development of new generations of anticancer agents based on metals other than platinum.[4–8]

In the last 2 decades there has been a growing interest in coordination and organometallic palladium compounds as potential alternative anticancer drugs,[9–11] inspired by its similar coordination chemistry to that of platinum. The good antiproliferative activity toward several cisplatin-sensitive and cisplatin-resistant tumor cell lines and their mode of action (which, in the few cases studied, appears sometimes quite different from that of cisplatin and its analogs) are the main reasons for the increasing popularity of palladium compounds as therapeutic agents.[12]

A critical aspect that initially discouraged the study of palladium complexes as potential anticancer agents was their higher kinetic lability compared to that of their platinum congeners. The rapid hydrolysis of palladium-ligand bonds generally results in the formation of very reactive species that are unable to reach the target biomolecules inside cancer cells. A possible strategy to reduce or even overcome this limitation was the use of polydentate and/or bulky monodentate ligands strongly bound to the metal center.[12] Among these, palladium organometallic compounds have come to the fore for their good stability due to the presence of at least one strong palladium-carbon bond.

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Cell Reports Physical Science Review

In this review, we propose an overview of the most important palladium organometallic compounds as potential anticancer agents studied in the last 6–7 years. Particular attention will be paid to the structure/activity relationships and mode of action proposed.

Palladacyclic complexes

Cyclopalladated imines and tetranuclear cyclopalladates

Among the organopalladium complexes most studied as potential anticancer agents, the palladacyclic species play a prominent role. These compounds, due to the presence of multidentate ligands and at least one palladium-carbon bond, are generally stable both in common organic solvents and in the physiological environment. In the review published by Fairlamb and Kapdi[12] at the beginning of 2014, numerous examples of palladium complexes belonging to this category were reported, some of which have an interesting antitumor activity on both cisplatin-sensitive and cisplatin-resistant cell lines (see Figure 1A).

In the same year, Albert and coworkers[13,14] reported two interesting contributions concerning the synthesis and a detailed analysis of the biological activity of mono- and dinuclear *endo* cyclopalladated benzophenone imines (see Figure 1B). In particular, cyclopalladates bearing a hydrogen on the nitrogen atom (**1a–b** and **2a–b**) showed good antiproliferative activity toward MCF-7 and MDA-MB-231 cancer cells, with the mononuclear complexes **1a–b** (half-maximal inhibitory concentration at 72 h [$IC_{50}^{(72h)}$] = 4.0–4.1 μM [MCF-7] and 1.1 μM [MDA-MB-231]) being ~4 times more active than cisplatin ($IC_{50}^{(72h)}$ = 19 μM [MCF-7] and 7 μM [MDA-MB-231]) and more active than the corresponding dinuclear cyclopalladates **2a–b** ($IC_{50}^{(72h)}$ = 11–14 μM [MCF-7] and 13–15 μM [MDA-MB-231]). Unlike the complexes described above, those containing alkyl or aryl substituents at the nitrogen atom (**3–10**) were found to be inactive ($IC_{50}^{(72h)}$ > 100 μM) or moderately active ($IC_{50}^{(72h)}$ = 20–50 μM) on the same tumor lines. The *n*-octanol/water partition coefficient values determined for the tested compounds appear to suggest that an accurate balance between hydrophilicity and lipophilicity may be one of the key factors explaining this different antitumor activity. The most active compounds **1–2**, due to their N-H function, have a logP = 5.7–9.6, whereas inactive compounds **3–10** ($IC_{50}^{(72h)}$ > 100 μM) are instead the most lipophilic among those tested (logP = 12–14). Interestingly, complex **1b**, in addition to exhibiting high accumulation in cancer cells, was found to be less cytotoxic than cisplatin against human umbilical vein endothelial cells (HUVEC) in starving (quiescent) and under normal cell culture conditions ($IC_{50}^{(72h)}$ = 49 versus 16 μM [$HUVEC_{starving}$] and 29 versus 18 μM [$HUVEC_{normal}$]). Finally, DNA migration and cathepsin B inhibition experiments suggested that these biomolecules are not the primary target for this category of compounds. Three years later, the same group reported the anticancer, antibacterial, and antioxidant activities of *endo* and *exo* cyclopalladated (E)-*N*-([1,1′-biphenyl]-2-yl)-1-mesitylmethanimines (**11a–b**).[15] Given their structural similarity with the cyclopalladated benzophenone imines **1–10**, they were also tested against MCF-7 and MDA-MB-231 cells. The *exo* isomer **11a** exhibited cytotoxicity toward tumor cells in the micromolar range ($IC_{50}^{(72h)}$ = 13–17 μM) and 7 times lower cytotoxicity toward BJ normal cells compared to cisplatin ($IC_{50}^{(72h)}$ = 57 versus 8 μM). Conversely, the *endo* isomer **11b** showed low activity on all of the examined cell lines ($IC_{50}^{(72h)}$ > 42 μM). Moreover, both structural isomers showed a noticeable antibacterial and antioxidant activity and, analogous to the cyclopalladated benzophenone imine derivatives, do not induce either alteration of the DNA tertiary structure or significant inhibition of cathepsin B.

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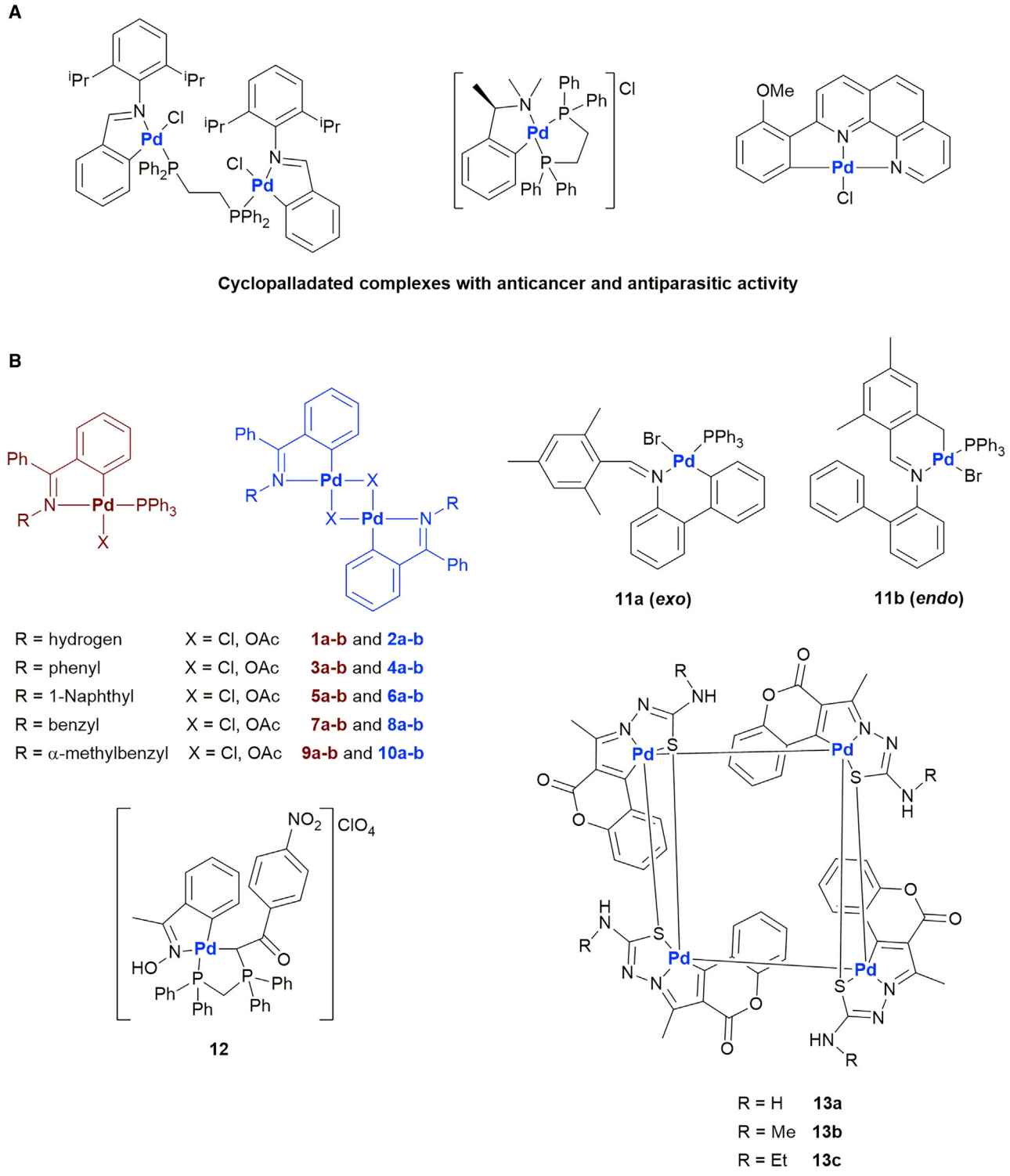

Figure 1. Examples of palladacyclic complexes with interesting anticancer properties

(A) Palladacyclic complexes with remarkable anticancer activity published before 2014.

(B) Palladacyclic complexes studied by Albert (**1–11**), Samiee (**12**), and Prabhakaran (**13a–c**).

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In 2019, Samiee et al.[16] reported the catalytic and anticancer activity of **12**, which contains a palladacyclic fragment similar to that published by Albert et al.[15] in 2014. An unsymmetrical phosphorus ylide ligand occupies the two remaining coordination sites of this oxime palladacycle. The antiproliferative activity tests toward HT-29 and A549 cell lines proved its good cytotoxicity ($IC_{50}^{(72h)}$ = 9 and 10 μM, respectively). Fluorescence studies (dual acridine orange/ethidium bromide [AO/EB] double staining) and morphological characterization of treated cancer cells showed the ability of **12** to induce cell death via apoptosis.

In the same year, Prabhakaran[17] reported the synthesis of tetranuclear palladacyclic complexes **13a–c**, which showed cytotoxicity in the 5–7 μM range (48 h) against HepG2 and HT-29 cancer cells. Interestingly, the same complexes exhibited low cytotoxicity toward HaCaT human normal keratinocytes ($IC_{50}^{(48h)}$ > 40 μM). Fluorescence microscopic analyses (AO-EB and DAPI staining assays) and the classical annexin-V method suggested an apoptotic cell death and significant morphology changes such as nuclear shrinkage and chromatin condensation. Furthermore, the authors observed an intercalative mode of binding of complexes **13a–c** with circulating tumor-DNA (CT-DNA), which was further confirmed by competitive DNA binding studies and DNA viscosity measurements. Finally, all of the complexes strongly interact with albumins (BSA and human serum albumin [HSA]), with binding constants in the range (1.2–9.9) • 10^4 M^{-1}, as evidenced by detailed 3-dimensional (3D) fluorescence studies.

Dinuclear palladacyclic complexes with bridging diphosphine ligands

In the field of polynuclear complexes, Karami and coworkers[18] reported between 2014 and 2017 dinuclear cyclopalladates (**14a–b** and **15a–b**) with remarkable anticancer properties (see Figure 2). In particular, complexes **14a–b**, which contain DPPF (1,1′-bis(diphenylphosphine)-ferrocene) and BPP (1,3-bis(4-pyridyl)propane) as bridging diphosphines, exhibited good cytotoxicity against JURKAT and SKOV3 cell lines ($IC_{50}^{(48h)}$ = 2–7 μM).[18] Both compounds demonstrated lower cytotoxic activity toward normal peripheral blood mononuclear cells (PBMCs) ($IC_{50}^{(48h)}$ = 39 and 31 μM, respectively) and a significant binding affinity with DNA and BSA. The higher cytotoxicity and selectivity of **14a** compared to **14b** is attributed to its greater affinity with DNA (K_b = (2.32 and 1.11) • 10^5 M^{-1}, respectively), which is due to intercalation of the diphenylphosphine moiety and the hydrophobic interaction of the amine residues.

Similar binding affinities with DNA and BSA were observed for compounds **15a–b**, which bear DPPE (1,2-bis(diphenylphosphino)ethane) as a bridging ligand and a different palladacyclic architecture.[19] These compounds have been tested against HeLa, HT-29, K562, and MCF-7 tumor cells with $IC_{50}^{(72h)}$ in the range 2.5–8.5 μM.

Complex **16**, characterized by a dppf linker, was investigated by Bincoletto and coworkers[20] as an antimelanoma agent toward metastatic (Tm5) and nonmetastatic ($4C11^-$) cells ($IC_{50}^{(24h)}$ = 10 and 6.5 μM, respectively).[20] This compound, which has been extensively studied since the early 2000s,[21] induces apoptosis and late apoptosis involving the lysosomal mitochondrial axis. This pathway is characterized by lysosomal membrane permeabilization (LMP), cathepsin B activation, and increased Bax protein levels following its translocation to mitochondria. Subsequently, the alteration of the mitochondrial membrane potential and the caspase-3 activation result in cell death. Interestingly, the observed high p62 protein level suggested a blocked autophagy. In support of this hypothesis, the treatment of metastatic and nonmetastatic melanoma cells with 3-methyladenine, a well-known

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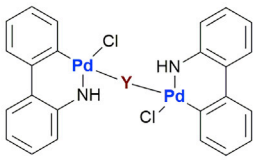
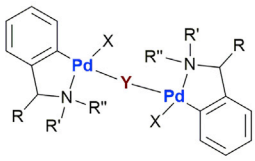
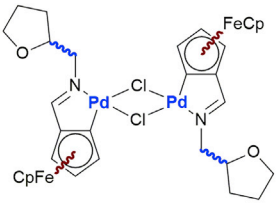


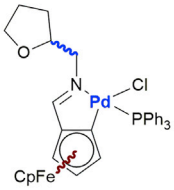
Y = μ-dppf **14a**
Y = μ-bpp **14b**



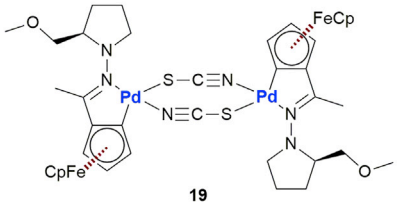
R = H, R' = H, R'' = Et, X = Cl, Y = μ-dppe **15a**
R = H, R' = H, R'' = Et, X = Br, Y = μ-dppe **15b**
R = Me, R' = Me, R'' = Me, X = Cl, Y = μ-dppf **16**



(Rp, S, S, Rp) **17a**
(Sp, S, S, Sp) **17b**
(Sp, R, R, Sp) **17c**
(Rp, R, R, Rp) **17d**



(Rp, S) **18a**
(Sp, S) **18b**
(Sp, R) **18c**
(Rp, R) **18d**



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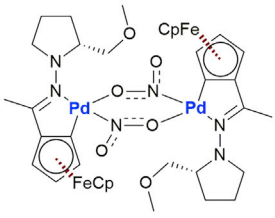

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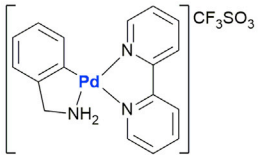
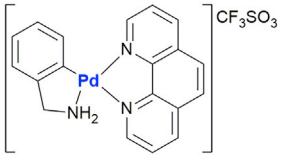


21a



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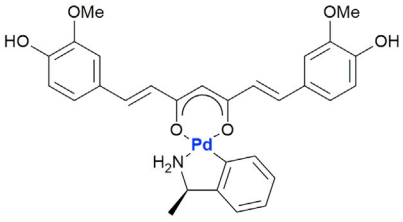

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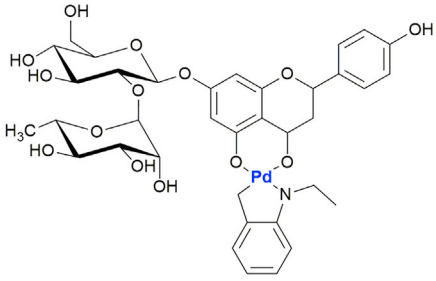
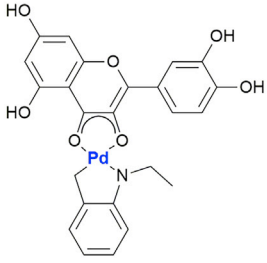
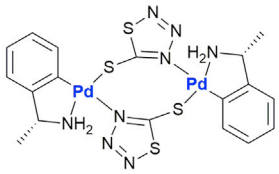


22a



22b



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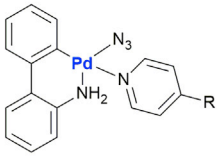
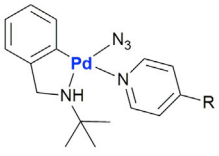
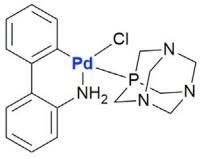


R = -$CONH_2$ **25a**
R = -NMe_2 **25b**



R = -$CONH_2$, **26a**
R = -NMe_2 **26b**



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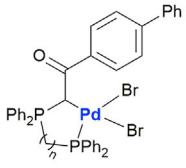
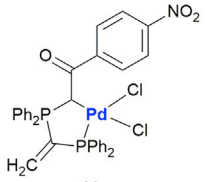


n = 1, 2 **28a-b**



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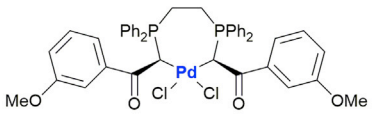

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inhibitor of the initial stage of autophagy, increased the activity of complex **16**. Therefore, it can be inferred that autophagy could be one of the key factors in the mechanism of melanoma cells resistance.

Mono- and dinuclear ferrocene cyclopalladates

In the last 3 years, Zhao and coworkers[22] have synthesized and investigated the antitumor activity of some mononuclear and dinuclear ferrocene cyclopalladated compounds (**17–20**). All of the chiral complexes **17a–d** and **18a–d** were found to be more active than cisplatin against MCF-7, HCT-116, MDA-MB-231, and HeLa cells ($IC_{50}^{(72h)}$ = 0.4–9.2 μM [**17–18**] and 6.5–22.2 μM [cisplatin]), and, among them, the mononuclear species **18a–d** exhibited greater cytotoxicity than their dinuclear congeners **17a–d** ($IC_{50}^{(72h)}$ = 0.4–8.0 μM [**18a–d**] versus 1.3–9.2 μM [**17a–d**]).[22] Complexes **19** and **20**, bearing thiocyanate and nitrite ligands, proved to be extremely active toward HepG2, 4T1, and KGN cell lines, with IC_{50} values on average 10-fold lower than those of cisplatin ($IC_{50}^{(72h)}$ = 0.1–0.7 μM [**19–20**] versus 5–14 μM [cisplatin]).[23,24]

Cyclopalladates with C^N, C^P, and C^C architectures

Between 2017 and 2019, Karami[25–28] reported a series of palladacycles (**21–26**), which showed, among the various cell lines tested, a moderate/good antiproliferative activity against MCF-7 cancer cells ($IC_{50}^{(48h)}$ = 3–50 μM). In many cases, this cytotoxicity is accompanied by a lower activity toward PBMC normal cells ($IC_{50}^{(48h)}$ > 100 μM). All of the tested compounds showed a strong affinity with DNA (via intercalation) and BSA (via Sudlow's site 1). Interestingly, within each subcategory of complexes, the trend of antitumor activity is closely related to the experimentally determined binding constant of the complex with CT-DNA.

Using the same approach, Lighvan and Khonakdar[29] recently synthesized and investigated the palladacyclic compound **27** bearing the 1,3,5-triaza-7-phosphaadamantane (PTA) ligand. This complex strongly interacted with DNA and BSA and showed good cytotoxicity against MCF-7 and JURKAT cancer cells ($IC_{50}^{(48h)}$ = 35 and 51 μM, respectively).

In 2018, Sabounchei and colleagues[30] reported the synthesis of organopalladium complexes bearing unsymmetrical phosphorus ylides (**28a–b**). *In vitro* tests proved their high tumor-specific cytotoxicity against A2780 cells ($IC_{50}^{(24h)}$ = 16–21 μM). The same compounds are, however, significantly less active against U87 and H12299 cell lines ($IC_{50}^{(24h)}$ = 22–51 and >61 μM, respectively). It is worth mentioning that the same behavior was observed for their platinum congeners. Moreover, it was demonstrated that the higher cytotoxicity of **28a** compared to **28b** is associated with its higher antioxidant activity evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. A year later, the same authors[31] discussed the synthesis and biological activity of **29**, which contains a palladacyclic fragment similar to **28a** and 2 coordinated chlorides instead of 2 bromides. Compound **29** is active against the A2780 line, as well as against HT-29 cells ($IC_{50}^{(24h)}$ = 10 and 9 μM, respectively). In the same year, Sabounchei[32] observed the high tumor-specific cytotoxicity against KB cells ($IC_{50}^{(24h)}$ = 18 μM) of an interesting palladium complex (**30**) bearing a C,C-chelating phosphorus ylide ligand.

Figure 2. Recent dinuclear and mononuclear complexes as promising anticancer agents

Dinuclear palladacyclic complexes with bridging diphosphine ligands, mono- and dinuclear ferrocene cyclopalladates, and cyclopalladates with C^N, C^P and C^C architectures.

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Palladacyclopentadienyl complexes

An intriguing category of palladium compounds whose reactivity and catalytic activity was known, but the remarkable antitumor activity remained unexplored before 2019, is represented by palladacyclopentadienyl complexes.[33,34] The palladacyclopentadienyl framework is a particularly stable structure since it can be quickly attacked only by strong oxidants such as halogens or inter-halogens,[35–38] which are not usually present in biological environments.

Taking advantage of these reactivity studies and the promising results obtained by Hashmi[39] against HeLa and Caco-2 cancer cells treated with chiral 5-palladatricyclo [4.1.0.02,4]heptanes, Visentin and Scattolin[40] synthesized in 2019 a wide range of complexes (**31–34a–c**) in which the palladacyclopentadienyl motif is combined with strong donor spectator ligands such as xanthine-based *N*-heterocyclic carbenes (NHCs) and phosphines (PPh_3 and PTA) or 2,6-dimethylphenyl isocyanide (DIC) (see Figure 3). The goal was to prepare very stable complexes even in a biological environment that therefore could behave as structural compounds, according to the smart metallodrugs classification proposed by Alessio[41] in 2009. Furthermore, NHC ligands with a purinic framework were used in the hope that the natural imprint of the moiety could make these palladium complexes more compatible with the biological matrix. The results obtained toward A2780 and A2780*cis* cancer cells showed a substantial inactivity of the polymeric $[PdC_4(COOMe)_4]_n$, but an antitumor activity comparable or even better than cisplatin ($IC_{50}^{(72h)}$ [cisplatin] = 0.6 μM [A2780] and 6 μM [A2780*cis*]) for most of the final compounds tested (**31–34a–c**).[40] In particular, compounds containing the scarcely hindered ligands PTA and DIC (**33–34**) were the most active species on both lines ($IC_{50}^{(72h)}$ = 0.56–4.3 μM [A2780] and 0.62–2.1 μM [A2780*cis*]). Interestingly, all of the compounds have very similar IC_{50} values between the 2 lines, suggesting a different mechanism of action than cisplatin. Furthermore, compounds containing PTA (**33a–c**) induce significant apoptosis and have been found to be poorly cytotoxic against MRC-5 lung fibroblasts ($IC_{50}^{(72h)}$ > 100 μM), indicating a certain selectivity toward cancer cells.

A more detailed and systematic study on the antitumor activity of palladacyclopentadienyl complexes was developed by the same authors, examining 5 different categories of ligands (chelating bisNHCs **35a–b**, monodentate bisNHCs **36a–c**, mixed NHC/PPh_3 or NHC/DIC **37–38**, hemilabile C-S or C-N **39a–b**, and labile N-S or N-P **40–41**) and their effects against 6 cancer cell lines (A2780, A2780*cis*, OVCAR5, A549, A375, and DLD-1) and MRC-5 normal cells.[42]

Antiproliferative activity data proved that the most cytotoxic complexes are those containing ancillary ligands firmly anchored to the metal center (**35–38**). Among these, the biscarbene complexes **35–36** were found to be stable over time even in the presence of an excess of glutathione (GSH), which is one of the most abundant nucleophiles present in the cellular environment. An in-depth study was carried out on **35a**, one of the compounds with the highest cytotoxicity against all ovarian cancer cell lines ($IC_{50}^{(96h)}$ = 0.039 μM [A2780], 2.8 μM [A2780*cis*], 0.30 μM [OVCAR5]) and at the same time poorly active toward MRC-5 normal cells ($IC_{50}^{(96h)}$ > 100 μM). A kinetic analysis showed that **35a** acts primarily on DNA and only subsequently induces alterations to the other cellular compartments (intrinsic apoptosis). The most probable hypothesis for the mechanism of action of this compound is a strong non-covalent interaction with DNA, similar to many organic anticancer agents (e.g., doxorubicin).

With the aim of studying the reactivity of palladacyclopentadienyl complexes bearing picolyl-NHCs toward iodine, Visentin and colleagues[43] isolated a novel

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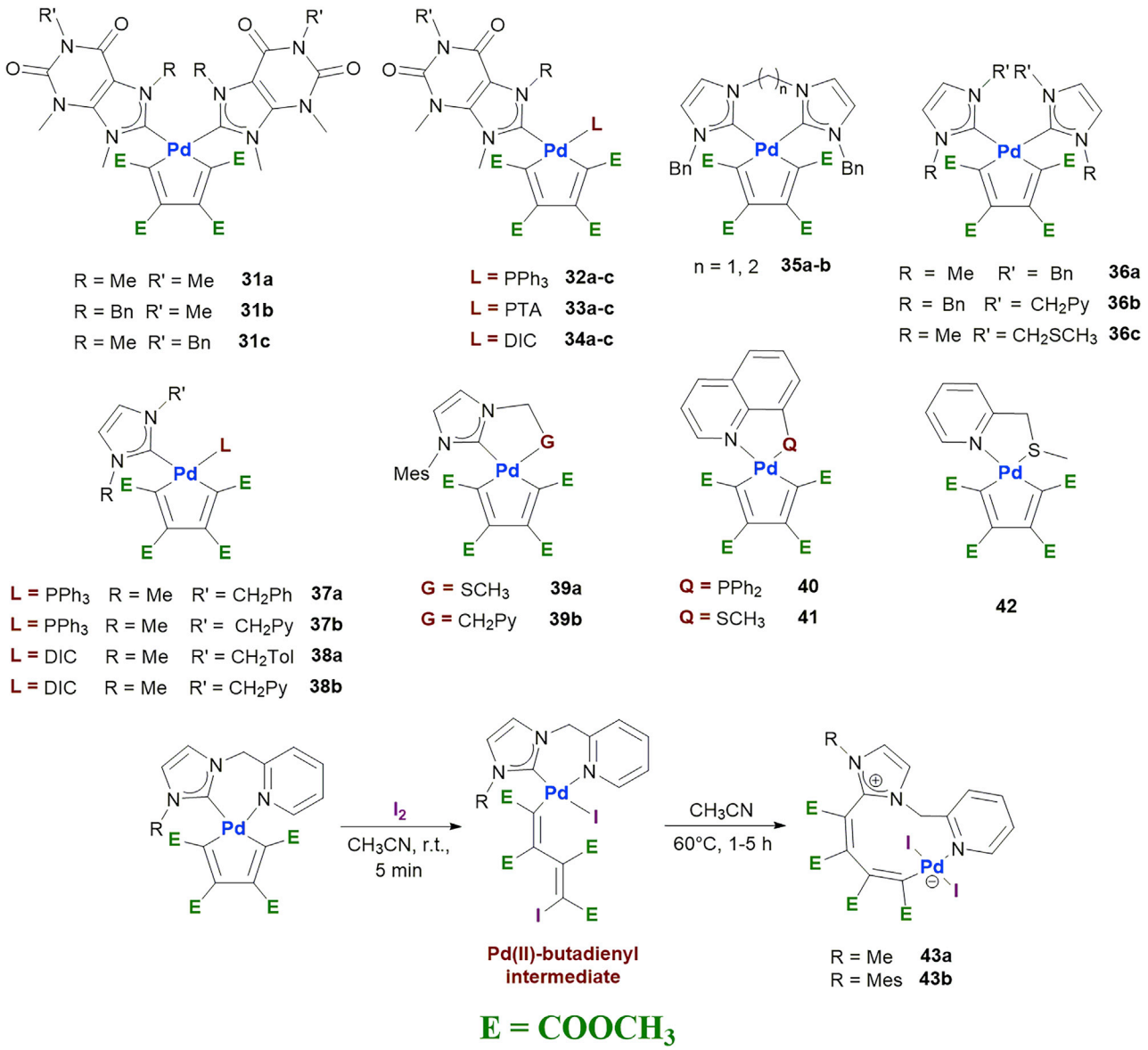

Figure 3. Palladacyclopentadienyl complexes with promising anticancer activity
Palladacyclopentadienyl complexes and their zwitterionic derivatives.

and unique category of organopalladium compounds. The expected opening of the palladacyclopentadienyl fragment to form the classical σ-butadienyl derivatives curiously evolves over time in the **43a–b** species, which present a 10-membered coordinative ring. Both compounds are extremely stable in solution even at relatively high temperatures (60°–80°C) and in the presence of oxygen and moisture. These unusual zwitterionic species were tested against 8 tumor lines, showing the higher cytotoxicity of **43b** compared to cisplatin and **43a** ($IC_{50}^{(96h)}$ [**43b**, **43a**, cisplatin] = 0.51, 5.8, 0.81 μM [A2780], 1.12, >100, 43 μM [A2780*cis*], 4.4, 6.8, 5.2 μM [OVCAR5], 7.0, >100, 17.7 μM [SKOV3], 4.6, >100, 47 μM [SKOV3*cis*], 3.5, >100, 6 μM [A549], 4.4, >100, 19 μM [DLD-1], 0.3, 2, 4.7 μM [A375]).[44] Furthermore, both palladium complexes were found to be substantially inactive toward MRC-5 normal cells ($IC_{50}^{(96h)}$ [**43b**, **43a**, cisplatin] = >100, >100, 14 μM).

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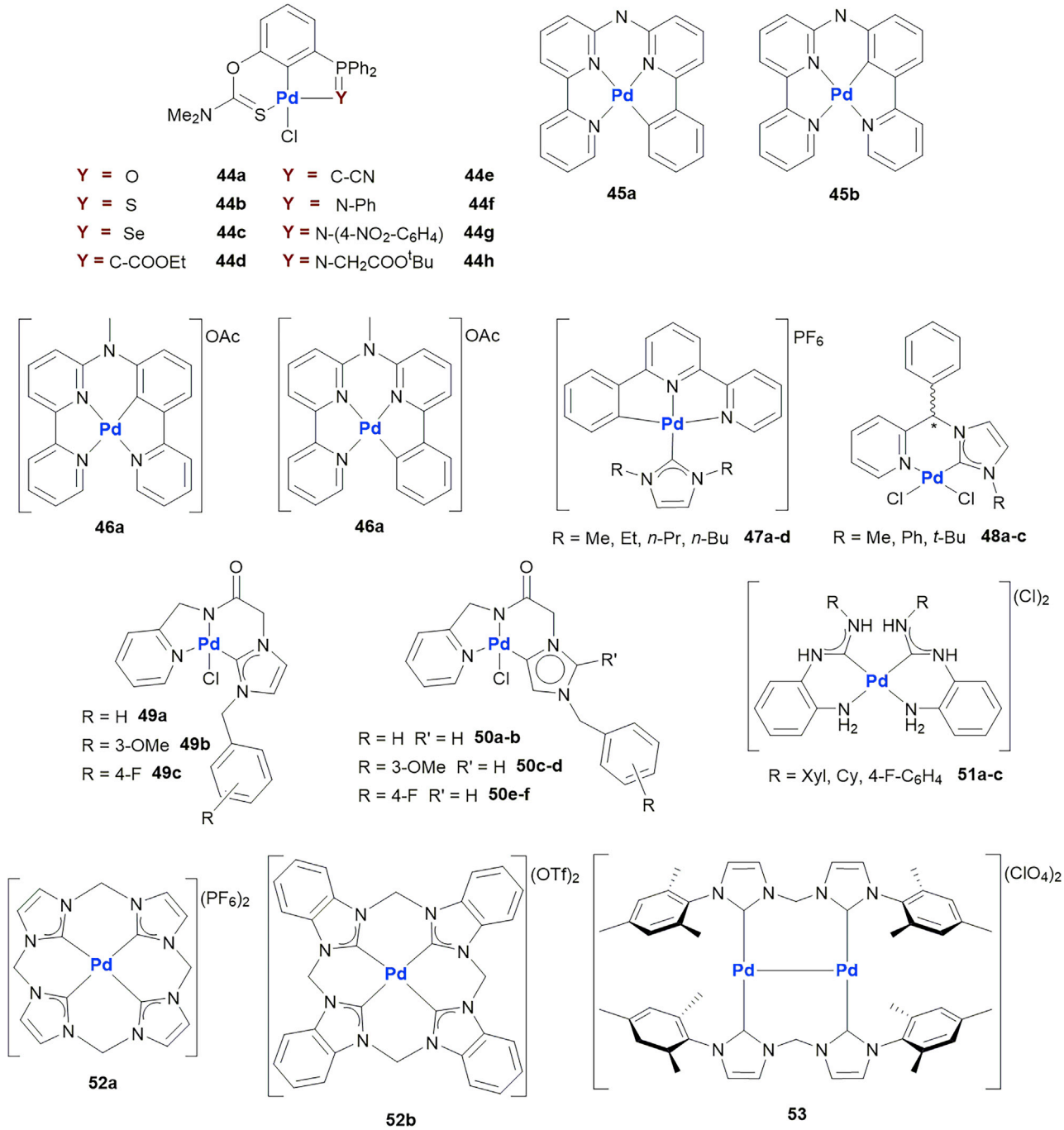

Figure 4. Cyclopalladates bearing carbene ligands and other tested palladacyclic complexes

Cyclopalladates bearing P(V)-ancillary ligands, photoactive cyclopalladates, and palladacyclic complexes bearing *N*-heterocyclic carbene ligands.

Cyclopalladates bearing P(V)-ancillary ligands

Aleksanyan[45] recently reported the synthesis of palladacyclic complexes with hybrid pincer ligands bearing a thiocarbamate arm and different P(V)-ancillary donor groups: phosphine oxide (**44a**), phosphine sulfide (**44b**), phosphine selenide (**44c**), phosphonium ylide (**44d–e**), and phosphine imide (**44f–h**) (see Figure 4). Preliminary *in vitro* tests against HCT-116, MCF-7, and PC-3 cancer cells exibited the following

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trend of antitumor activity: phosphine imide (**44f–g**) > phosphine sulfide/selenide (**44b–c**) > phosphonium ylide (**44d**) ($IC_{50}^{(48h)}$ [**44f–g**, **44b–c**, **44d**] = 2.5–7.5, 18–24, 46 μM [HCT-116], 42–48, 56 μM [MCF-7], 3–8, 18–20, 52 μM [PC-3]). Unfortunately, all of the examined compounds had comparable activity against cancer cells and HEK293 normal cells ($IC_{50}^{(48h)}$ = 4–55 μM).

Photoactive cyclopalladates

Some neutral and cationic complexes bearing polypyridyl ligands synthesized by Bonnet and colleagues[46] between 2019 and 2020 have received considerable interest. In their first report, they described the quite different photophysical properties of the 2 isomers **45a** and **45b**, with the former showing good absorbance in the blue region and excellent singlet oxygen quantum yield (0.89) and the latter having low absorption and low singlet oxygen quantum yield (0.38). Both compounds were tested toward A549 and A431 cancer cell lines, in the dark and in the presence of blue light (455 nm), using an exposure time and intensity that by themselves have no effect on cell growth. The antiproliferative activity data indicated the comparable cytotoxicity of the 2 compounds in the dark ($IC_{50}^{(48h)}$ [**45a–b**] = 12 and 8 μM [A549], 20 and 14 μM [A431]). On the contrary, after blue light activation, complex **45a** showed a 4- or 13-fold increase in cytotoxicity against A431 and A549 cells, whereas **45b** did not show significant changes ($IC_{50}^{(48h)}$ [**45a–b**] = 0.9 and 6 μM [A549], 5 and 10 μM [A431]). The difference in photocytotoxicity between the two coordination isomers can offer important insights into the numerous groups dealing with photodynamic therapy (PDT).

In another recent report, Bonnet and colleagues[47] showed that some monocationic palladacyclic complexes (**46a–b**) can also ensure, despite their apparent lower lipophilicity compared to neutral analogs, a high cellular uptake owing to their ability to self-assemble into soluble supramolecular nanorods when placed in aqueous solutions. These aggregates showed π-π stacking and metallophilic Pd···Pd interactions and are stabilized in the cell medium by serum proteins. These protein-stabilized self-assembled nanorods guarantee a high cellular uptake, which takes place via endocytosis (active uptake pathway). In the case of **46a**, the formation of the corresponding nanorods led to dramatically enhanced photodynamic properties under blue light irradiation. The remarkable photodynamic properties and high membrane penetration have also been confirmed on more complex models such as 3D tumor spheroids and in a mice tumor xenograft. Similar to **45a–b**, complexes **46a–b** had good antiproliferative activity in the dark toward A549 and A431 cells ($IC_{50}^{(48h)}$ [**46a–b**] = 2.2 and 2.7 μM [A549], 45 and 12 μM [A431]), with a notable increase in the cytotoxicity of **46a** after blue light irradiation ($IC_{50}^{(48h)}$ [**46a–b**] = 0.33 and 2.5 μM [A549], 4.8 and 7 μM [A431]).

Palladacyclic complexes bearing N-heterocyclic carbene ligands

As already reported in Figure 3, an important category of ancillary ligands that has allowed the development of promising organopalladium anticancer agents is represented by NHCs. The amazing ability of these ligands to stabilize most transition metal complexes,[48] combined with the availability of numerous synthetic routes for the preparation of M-NHC derivatives,[49] has made this class of σ-donor ligands extremely popular in catalysis,[50] material sciences,[51] and medicinal chemistry.[52] In this context, Che[53] reported in 2016 a class of palladacyclic complexes bearing different NHCs (**47a–d**). *In vitro* tests conducted on 6 different tumor lines (NCI-H1650, NCI-H460, MDA-MB-231, HeLa, A2780, and A2780*cis*) showed that these derivatives are much more active than compounds containing the same palladacyclic fragment combined with weaker ligands such as chloride, triphenylphosphine,

and *tert*-butyl isocyanide. Interestingly, such compounds, which are generally more cytotoxic than cisplatin in all of the tested lines ($IC_{50}^{(72h)}$ [**47a–d** and cisplatin] = 0.09–2.5 and 15.5 μM [NCI-H1650], 0.08–2.1 and 9.5 μM [NCI-H460], 0.5–0.9 and 21 μM [MDA-MB-231], 0.1–1.8 and 12 μM [HeLa], 0.2–1.7 and 1.5 μM [A2780], 0.5–2.2 and 31 μM [A2780*cis*]), are also extremely stable in the presence of biological thiols but unfortunately also moderately cytotoxic toward CCD-19Lu fibroblasts ($IC_{50}^{(72h)}$ = 11.8–32.2 μM). Furthermore, it was verified that they significantly inhibited tumor growth in a nude mice model. Finally, specific biochemical assays and proteomics data have proved that DNA is not the primary target of these complexes but rather that they cause various early effects such as mitochondrial dysfunction, antiangiogenic activity to endothelial cells, and inhibition of an epidermal growth factor receptor pathway.

In 2018, Cheow and colleagues[54] reported the synthesis and antitumor activity of racemic Pd-NHC complexes **48a–c**. The IC_{50} values of MCF-7, HCT-116, and H103 cell lines showed the low activity of compounds **48a** and **48c** ($IC_{50}^{(48h)}$ > 40 μM), while compound **48b** remained moderately active ($IC_{50}^{(48h)}$ = 14–37 μM). After resolving the racemic Pd-NHC complexes into the respective enantiomers, it was possible to demonstrate that the (R) isomers had a higher activity than their (S) analogs.

In 2015, Lee and Wang[55] disclosed the synthesis of interesting palladium(II) complexes (**49–50**) bearing tridentate ligands consisting of normal or abnormal NHCs, pyridine, and amidate donor moieties. *In vitro* tests against TOV21G, SW620, and NCI-H1688 cell lines showed a general higher activity of complexes bearing classical NHCs (**49a–c**) compared to those containing abnormal NHCs (**50a–f**). Among the compounds tested, **49b** is particularly promising since it exhibited cytotoxicity comparable to cisplatin toward TOV21G cancer cells ($IC_{50}^{(48h)}$ = 6.05 μM).

A series of C,N-chelate acyclic diaminocarbene (ADC) palladium(II) complexes (**51a–c**) were obtained by Boyarsky[56] in 2020 via metal-mediated coupling of 1,2-diaminobenzene to coordinated isocyanides. The authors assessed the antitumor potential of **51a–c** and their platinum congeners in 3 cancer cell lines (HT-29, MDA-MB-231, and MCF-7). Among these derivatives, the xylyl-substituted complex **51a** was the most active species ($IC_{50}^{(72h)}$ = 9.6–15.3 μM). This compound is able to induce significant apoptosis in MCF-7 cells and inhibition of the cell cycle in the G2/M phase, similar to cisplatin. Furthermore, spectroscopic and hydrodynamic techniques proved the strong affinity between **51a** and DNA due to the combination of electrostatic interaction and covalent binding in the major groove. This palladacyclic species, which is capable of establishing numerous hydrogen bonds, tends to form predominantly monofunctional adducts in the major groove of DNA as a result of the nucleophilic substitution of labile amino leaving groups by DNA nitrogen bases, which is in turn favored by the presence of strong *trans*-labilizing ADC ligands.

The interesting category of palladium(II) complexes bearing macrocyclic tetra-NHC ligands (**52a–b**) has recently been investigated by Kühn and coworkers[57] as potential anticancer agents. In particular, these compounds were tested against HeLa, MCF-7, and A2780*cis* cancer cells showing excellent activity in the micro- and sub-micromolar range, comparable to that of their platinum congeners and sometimes higher than cisplatin ($IC_{50}^{(48h)}$ (**52a–b**, platinum congeners and cisplatin) = 0.4–2.8, 0.5–2.9, and 2.8 μM (HeLa), 1.3, 0.2–1.7, and 4.7 μM (MCF-7), 0.1–1.9, 0.2–1.7, and 11 μM A2780*cis*). However, the corresponding nickel complexes were found to be poorly active or inactive. The remarkable luminescence properties of **52a–b** may be useful for studying their cellular uptake/distribution and to investigate their mechanism of action.

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Although most of the palladium complexes studied as potential anticancer agents present the metal center in the classical oxidation state +2, in 2020, Visentin and Scattolin[58] reported the first and detailed study of the antitumor properties of the fascinating palladium(I) dimer **53**, which bear only 2 bridging bisNHC ligands. Despite the high unsaturation of the two metal centers and the well-known poor stability of most palladium compounds in the oxidation state +1, this particular compound showed a surprising bench-top stability with an elevated air and moisture tolerance. Pd(I) dimer **53**, which can be described as a biradical singlet species, was tested against 8 different tumor lines, mainly of ovarian cancer, showing exceptional antiproliferative activity ($IC_{50}^{(96h)}$ = 0.025 and 1.9 μM [A2780, A2780*cis*], 1.4 and 3.0 μM [OVCAR5, OVCAR3], 0.38 μM [KURAMOCHI], 0.38 μM [A549], 2.8 μM [DLD-1], 3.5 μM [HeLa]). Moreover, this compound, in addition to being generally more active than cisplatin, was found to be substantially inactive toward MRC-5 normal cells. *Ex vivo* experiments on human tumoroids derived from high-grade serous ovarian cancer patients have confirmed the remarkable activity of **53** against this aggressive type of female tumor. In addition, the low cytotoxicity against normal liver organoids have confirmed the near invasiveness toward non-cancerous cells. Finally, specific immunofluorescence assays have shown the ability of this Pd(I) dimer to induce mitochondrial dysfunction already in the first minutes/hours of cell treatment (alteration of the mitochondrial membrane potential and cytochrome *c* release). In contrast, the cells treated with **53** do not show DNA damage after 12 h, suggesting that DNA is unlikely to be its major molecular target.

Acyclic palladium complexes bearing NHC ligands

In 2007, the anticancer activity of acyclic NHC-palladium complexes was examined for the first time by Ghosh, Panda, and coworkers.[59] The choice of *trans*-[(NHC)$Pd(py)Cl_2$] and *trans*-[$(NHC)_2PdCl_2$] complexes was based on previous work that reported the high activity of platinum *trans*-bispyridine complexes and on the well-known antiproliferative properties of Pd-pyridine and Pd-amine complexes. Bis-NHC complex **54a** (see Figure 5) has shown remarkable antiproliferative activity at low micromolar concentrations toward 3 different cancer cells ($IC_{50}^{(24h)}$ = 4 μM [HeLa], $IC_{50}^{(48h)}$ = 1 μM [MCF-7] and $IC_{50}^{(16h)}$ = 0.8 μM [HCT-116]), superior to benchmark cisplatin ($IC_{50}^{(24h)}$ = 8 μM [HeLa], $IC_{50}^{(48h)}$ = 15 μM [MCF-7] and $IC_{50}^{(16h)}$ = 16 μM [HCT-116]). That work can be considered pioneer in acyclic Pd-NHC complexes.

A later study was performed by Haque and colleagues[60] in 2013 and was dedicated to complexes of similar structure with different NHCs. The antiproliferative activity against HCT-116 cells of one of the synthesized complexes (**54b**) is high ($IC_{50}^{(72h)}$ = 6.6 μM), although lower than that of the previously reported **54a**. Interestingly, if the benzyl arm is replaced with the allyl group, then it leads to the formation of *cis*-complex **55**. However, such modification, rather than improving, decreased the cytotoxicity ($IC_{50}^{(72h)}$ = 26.5 μM) making this compound less active than cisplatin. Compounds **54c** and **56**, which bear methyl groups, showed no activity toward HCT-116 cells.[61]

Further investigations on the antitumor activity of complexes of the type [$(NHC)_2PdX_2$] were carried out by changing counterion and NHC structure to the benzimidazolium core.[62] Complex **57a** showed poor antiproliferative activity against HCT-116 cells ($IC_{50}^{(72h)}$ = 102.3 μM). Curiously, complex **58** with only 1 benzimidazolylidene as a spectator ligand was 2 times more active ($IC_{50}^{(72h)}$ = 51.5 μM). Interestingly, benzyl isomers **57b–c** again proved more active ($IC_{50}^{(72h)}$ = 16.3 and 21.4 μM, respectively) than their allyl congeners.

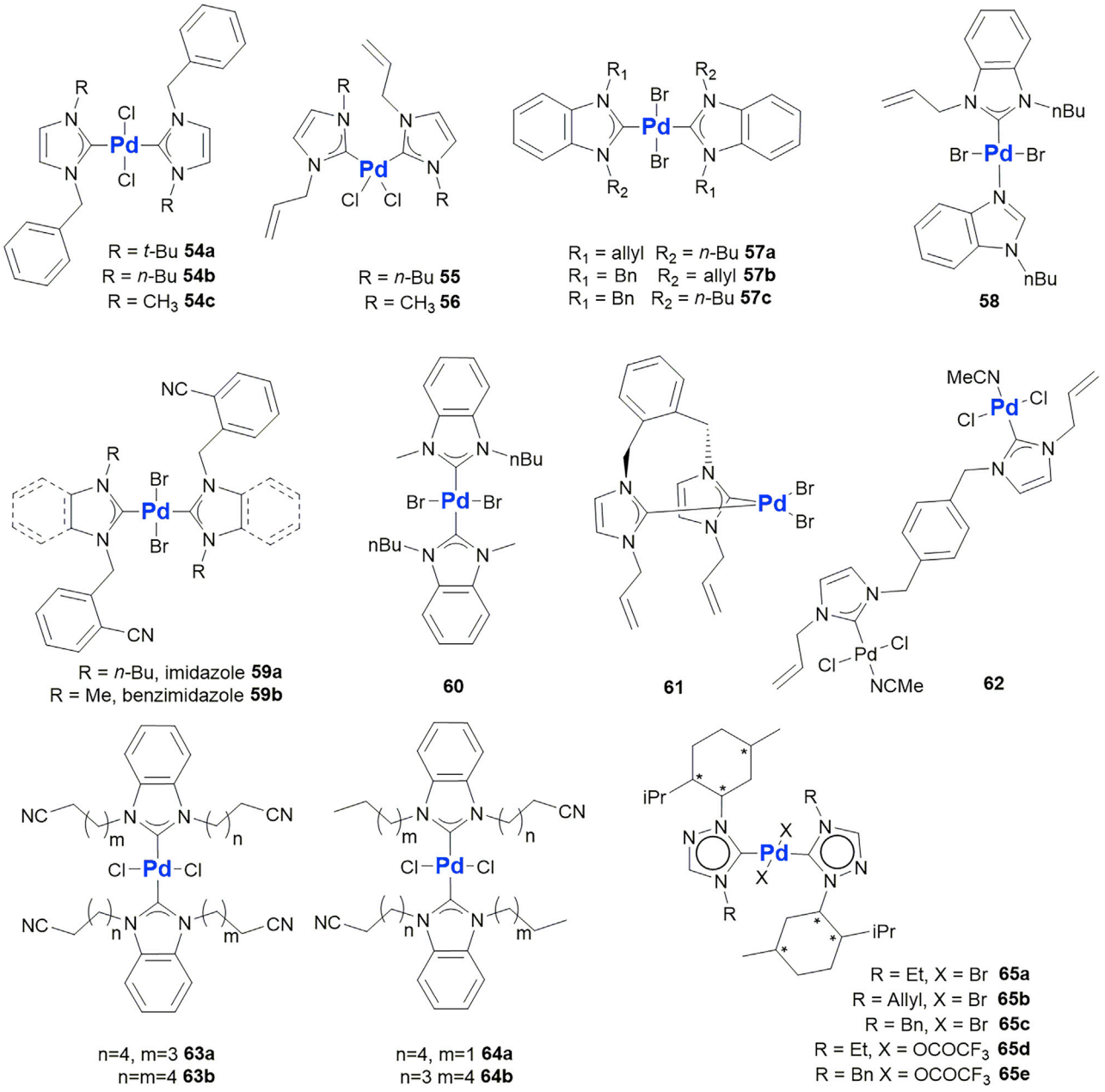

Figure 5. Bis-NHC palladium complexes examined for their antitumor properties

Recent examples of acyclic palladium complexes bearing NHC ligands tested toward different cancer cell lines.

In 2018, in-depth computational investigations of complexes **57b–c** and their silver analogs was carried out by Sayin.[63] Molecular docking using 3 model proteins, 1BNA, 1JNX, and 2ING, was performed. Sayin demonstrated that **57b** interacts better with 1JNX than with its silver counterpart.

The synthesis and the study of behavior of compounds **59–62** have allowed us to better define the effect of the steric and electronic features of NHC ligands on the anticancer activity of this class of complexes.[64] In particular, the best antiproliferative activity toward HCT-116 cells was observed for complex **60** with a benzimidazole core and the smallest substituents on NHC ligands ($IC_{50}^{(72h)}$ = 16.3 μM). Nitrile

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functionalization was found to be adverse: **59a** was less active ($IC_{50}^{(72h)}$ = 117.9 μM) than unfunctionalized **57b**, and, although **59b** activity was slightly better ($IC_{50}^{(72h)}$ = 42.1 μM), it remained moderate.

Another class of chelate NHC ligands with two cores connected by a xylyl spacer was used to prepare a new family of Pd bis-NHC complexes. However, only the *ortho* isomer allowed the formation of the mononuclear **61**, whereas in the case of the *para* isomer, only the binuclear species **62** was obtained. Nevertheless, both complexes proved highly cytotoxic against the same colon cancer cell line (HCT-116) in low micromolar concentrations ($IC_{50}^{(72h)}$ = 5.8 μM for **61** and 1.3 μM for **62**).

In 2018, other new cyano-functionalized complexes **63–64** were described in two reports by Razali and coworkers.[65,66] In both studies, antiproliferative effects were demonstrated against MCF-7 cancer cells and compared to the benchmark organic drug tamoxifen. All of the complexes showed a cytotoxicity ($IC_{50}^{(72h)}$ = 13.9–25.5 μM) comparable to that of tamoxifen ($IC_{50}^{(72h)}$ = 11.2 μM).

To investigate the influence of chirality on the anticancer activity of palladium complexes, **65a–e** were synthesized by Kumar et al.[67,68] Asymmetric 1,2,4-triazole-based NHC ligands with one arm containing three stereocenters were used in this study. Therefore, it was possible to prepare 2 diastereomeric versions—(1S,2S,5R) and (1R,2R,5S)—of each complex. Activity of 5 pairs of complexes were tested against the MCF-7 cell line, and no significant difference on the IC_{50} values was observed. In any case, **65a** and **65d** showed a cytotoxicity ($IC_{50}^{(48h)}$ = 2.2–2.3 μM and 0.55–0.7 μM, respectively) higher than cisplatin ($IC_{50}^{(48h)}$ = 14.9 μM). Complex **65d** was also tested against HeLa cancer cells, resulting in an ~3-fold higher activity than cisplatin ($IC_{50}^{(48h)}$ = 2.3 versus 8.5 μM), whereas its effect was less marked (8- and 16-fold lower) toward non-cancerous cell lines. The anticancer activity of **65d** was attributed to the cell-cycle arrest in G2 phase leading to p53-dependent apoptosis.

Another class of palladium compounds whose behavior in biological environment was extensively studied in recent years, is that of PEPPSI (pyridine-enhanced precatalyst preparation stabilization) complexes. The first complex **66** was prepared by Ghosh[59] in 2007 along with bis-NHC congeners, but its poor activity discouraged further studies, and PEPPSI complexes were not tested for anticancer properties for almost a decade (see Figure 6). Only in 2016 and 2017 did Akkoç and Kayser[69,70] report 2 studies including palladium complexes **67–69**. These were tested against DLD-1 and MDA-MB-231 cancer cells and HEK 393T normal cells. Unfortunately, all of the complexes did not exhibit antitumor activity ($IC_{50}^{(72h)}$ > 200 μM). Even changing the counterion from Cl^- to Br^- (in general, this improves the efficiency of bis-NHC complexes) or introducing an OH group in one arm of the NHC ligand, the antiproliferative activity against HeLa, DLD-1, MDA-MB-231, and HepG2 cell lines[71] did not increase and $IC_{50}^{(72h)}$ values of both complexes **70a–b** are >200 μM.

Dandekar and Kapdi[72] reported 3 water-soluble NHC palladium complexes **71a–c**, which were tested against HeLa and A549 cell lines. While the activities of **71a–c** against HeLa were lower ($IC_{50}^{(24h)}$ = 103–223 μM) than that of cisplatin ($IC_{50}^{(24h)}$ = 27 μM), for A549 cells, opposite results were observed ($IC_{50}^{(24h)}$ = 62–73 μM for complexes and 277 μM for cisplatin).

The introduction of biologically active motifs into metal complexes with promising anticancer properties is a common approach to increase their performance. Therefore, palladium-NHC complexes **72a–d** equipped with a benzotriazole arm were

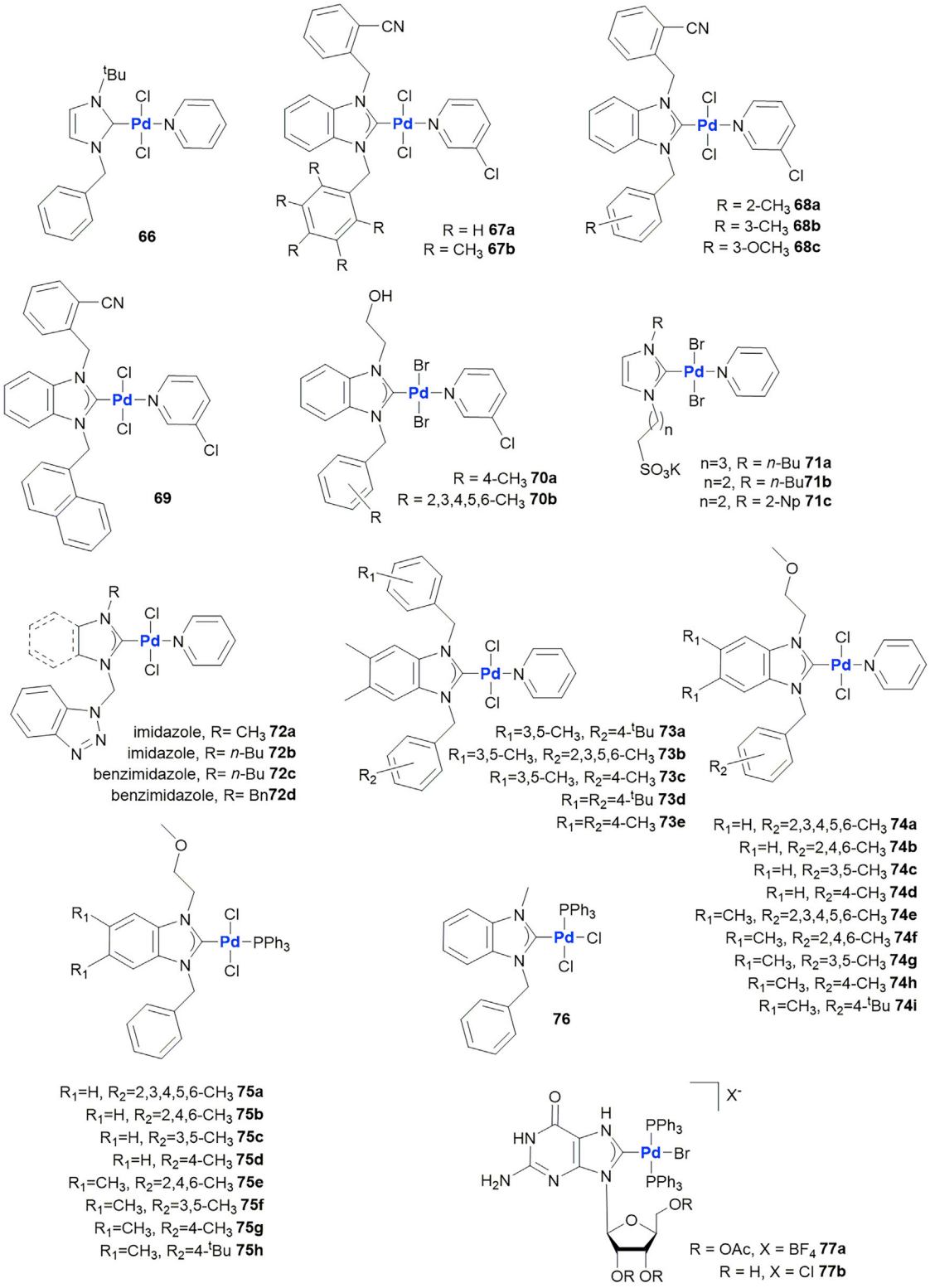

Figure 6. PEPPSI type and analogous complexes examined for their antitumor properties

An overview of PEPPSI-type complexes tested as potential anticancer agents.

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synthesized by Karatas and colleagues,[73] with the aim to exploit the well-known biological activity of benzotriazole. Their antiproliferative activity was evaluated against MCF-7 and Caco-2 cell lines, but the IC_{50} values obtained for all of the complexes were at least twice as high as cisplatin ($IC_{50}^{(24h)}$ [**72a–d** and cisplatin] = 162–376 and 76 μM [Caco-2], 192–530 and 81 μM [MCF-7]).

In 2019, Hamdi and coworkers[74] developed a series of 5 PEPPSI complexes **73a–e** bearing 5,6-dimethylbenzimidazole NHC ligands with a view to improving their catalytic activity. In this work, the antitumor activity was tested against MDA-MB-231, MCF-7, and T47D cell lines, obtaining encouraging results ($IC_{50}^{(72h)}$ = 6.9–22.4 μg/mL).

On the basis of these results, Hamdi and coworkers[75] screened palladium PEPPSI complexes with similar structures. Complexes **74a–i** with 5,6-dimethylbenzimidazole core and a functionalized benzyl arm were synthesized and tested *in vitro* against MCF-7 and MDA-MB-231 cancer cells. Intriguingly, the introduction of 2-methoxyehtyl substituent instead of the benzyl group significantly improved the cytotoxicity of the compounds. For all of the complexes, IC_{50} values against the MCF-7 cell line did not exceed 1.9 μM, with the best results for complexes **74f** and **74g** ($IC_{50}^{(72h)}$ = 0.675 and 0.518 μM, respectively). Similarly, IC_{50} values against MDA-MB-231 cells did not exceed 1.5 μM for all of the examined complexes, and the best result was achieved for **74h** ($IC_{50}^{(72h)}$ = 0.708 μM). In the same report, the authors explored the antitumor activity of palladium complexes bearing PPh_3 (**75a–h**), with interesting IC_{50} values against the aforementioned MCF-7 and MDA-MB-231 cancer cells ($IC_{50}^{(72h)}$ = 0.6–1.9 μM and 0.4–1.55 μM, respectively). The best activity was achieved for **75h** in both cases. The data obtained for mixed NHC-phosphine palladium complexes make them relevant for further studies.

Theoretical study, based on the PASS (Prediction of Activity Spectra for Substances) online tool, predicts that *cis*-complex **76** is likely to exhibit anticancer activity against melanoma at a high probability level.[76] In particular, the calculated probable activity (P_a) and probable inactivity (P_i) coefficients were 0.909 and 0.003, respectively, suggesting that this compound could be potential antimelanoma candidates.

The palladium complexes bearing NHC ligand derived from guanosine (**77a-b**) were tested against U251 cells.[77] Complex **77b** was found to exhibit $IC_{50}^{(48h)}$ values between 20 and 80 μM, but no further studies of similar compounds have been reported.

Palladium complexes bearing classical organometallic fragments

The poor intrinsic reactivity of palladacyclic species, described in the previous sections, appears to suggest that in the absence of reactive/labile co-ligands, they may behave predominantly as structural compounds in their interactions with the biotarget. Contrarily, alkyl/aryl, alkynyl, η^2-olefin, and η^3-allyl palladium derivatives are reactive species and their involvement in many typical reactions of organometallic chemistry (i.e., insertion, substitution, oxidative addition and reductive elimination, and nucleophilic/electrophilic attack on the coordinated ligand) is well known. This awareness has prompted some research groups to investigate the antitumor activity of palladium compounds bearing these reactive fragments and, in some cases, to propose a correlation between reactivity and cytotoxicity.

Palladium-aryl complexes

Following a chronological order, the first organopalladium complexes to be tested were aryl derivatives. In this respect, Ruiz and coworkers[78] reported in the early

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2000s the remarkable antitumor activity of complexes **78–80** toward HL-60 cancer cells (see Figure 7). In particular, all of the tested complexes, except **80a**, were found to be more active than cisplatin at a short incubation time (24 h), with IC_{50} values in the range of 4.7–11.3 μM ($IC_{50}^{(24h)}$ cisplatin = 15.6 μM). Flow cytometry experiments (annexin V-fluorescein isothiocyanate [FITC]) have shown that most compounds induce cell death by apoptosis. Moreover, atomic force microscopy data suggest that all complexes seem to modify the morphology of the pBR322 DNA in a fashion similar to that of cisplatin. Seven years later, Gouvea and coworkers[79] proved that the palladium-aryl complex **81** inhibited the growth of MDA-MB-435 cells, with an $IC_{50}^{(24h)}$ in the sub-micromolar range (0.1–0.5 μM), inducing significant alterations in the cell morphology. In 2018, Klein[80] successfully prepared some bis-aryl palladium complexes bearing 1,2-bis (diphenylphosphino)ethane (**82a–b**) or 1,5-cyclooctadiene (**83a–b**) ancillary ligands. Their antiproliferative activity was investigated against HT-29 and MCF-7 cell lines. Complexes **82a–b** and **83b** were found to be scarcely active ($IC_{50}^{(72h)}$ > 50 μM). Conversely, **83a** showed moderate cytotoxicity in the two lines, with $IC_{50}^{(72h)}$ values (26.0 and 47.4 μM) of an order of magnitude higher than those of cisplatin ($IC_{50}^{(72h)}$ cisplatin = 7 and 2 μM). Interestingly, similar results were obtained for their platinum congeners. Furthermore, *in vitro* tests toward leukemic L1210 cells and their cisplatin-resistant version (L1210/DDP) were performed for some platinum and palladium mono-aryl complexes. Comparison with the previously studied Pt(cod) complexes strongly suggest that the presence of at least one aryl ligand is crucial and, considering their high activity on cisplatin-resistant cells, a non-cisplatin-like mechanism of action may be at play. In particular, palladium mono-aryl complex **84** was found to be by far the most cytotoxic species, with $IC_{50}^{(72h)}$ values in the range of 0.6–0.9 μM in both investigated leukemic cell lines.

Palladium-alkynyl complexes

In 2017, Che and colleagues[81] described the synthesis of 2 classes of palladium(II)-alkynyl complexes bearing terpyridine (**85a–f**) or C^N^C (**86a–c**) ancillary ligands. The former did not show significant emission either in solution or in the solid state, whereas the [Pd(C^N^C)]-alkynyl complexes **86a–c** exhibited improved phosphorescence properties compared with their terpyridyl congeners, which is ascribable to the strong σ-donor character of the NHC ligands. In addition to the good photophysical properties, the presence of the 2 NHC units gives complexes **86a–c** high stability in an ammonium bicarbonate buffer solution even in the presence of reduced GSH. By combining photophysical data and stability tests, the authors decided to investigate the antitumor activity of **86a** and **86c** toward MDA-MB-231 cancer cells. Under dark conditions, both compounds displayed good antiproliferative and proapoptotic activities ($IC_{50}^{(48h)}$ (dark) = 1.5–2.8 μM). Interestingly, compound **86c**, which bears a pyrenylacetylide ligand, showed a 7.5-fold enhancement in cytotoxicity under visible light irradiation as a result of higher cellular ROS production ($IC_{50}^{(48h)}$ [light] = 0.2 μM). Conversely, complex **86a** did not show any phototoxic activity. Further experiments highlighted a low affinity between **86c** and DNA, suggesting a non-intercalative binding mode.

Palladium-olefin complexes

The only category of palladium(0) derivatives whose anticancer properties have been investigated so far is that of Pd(0)-η^2-olefin complexes. The nature of the Pd-olefin bond, the reactivity of this organometallic fragment toward different organic substrates, the catalytic activity of the corresponding complexes, and their thermodynamic stability depending on the type of coordinated olefin have been extensively studied in the last several decades.[82–91] However, with the exception

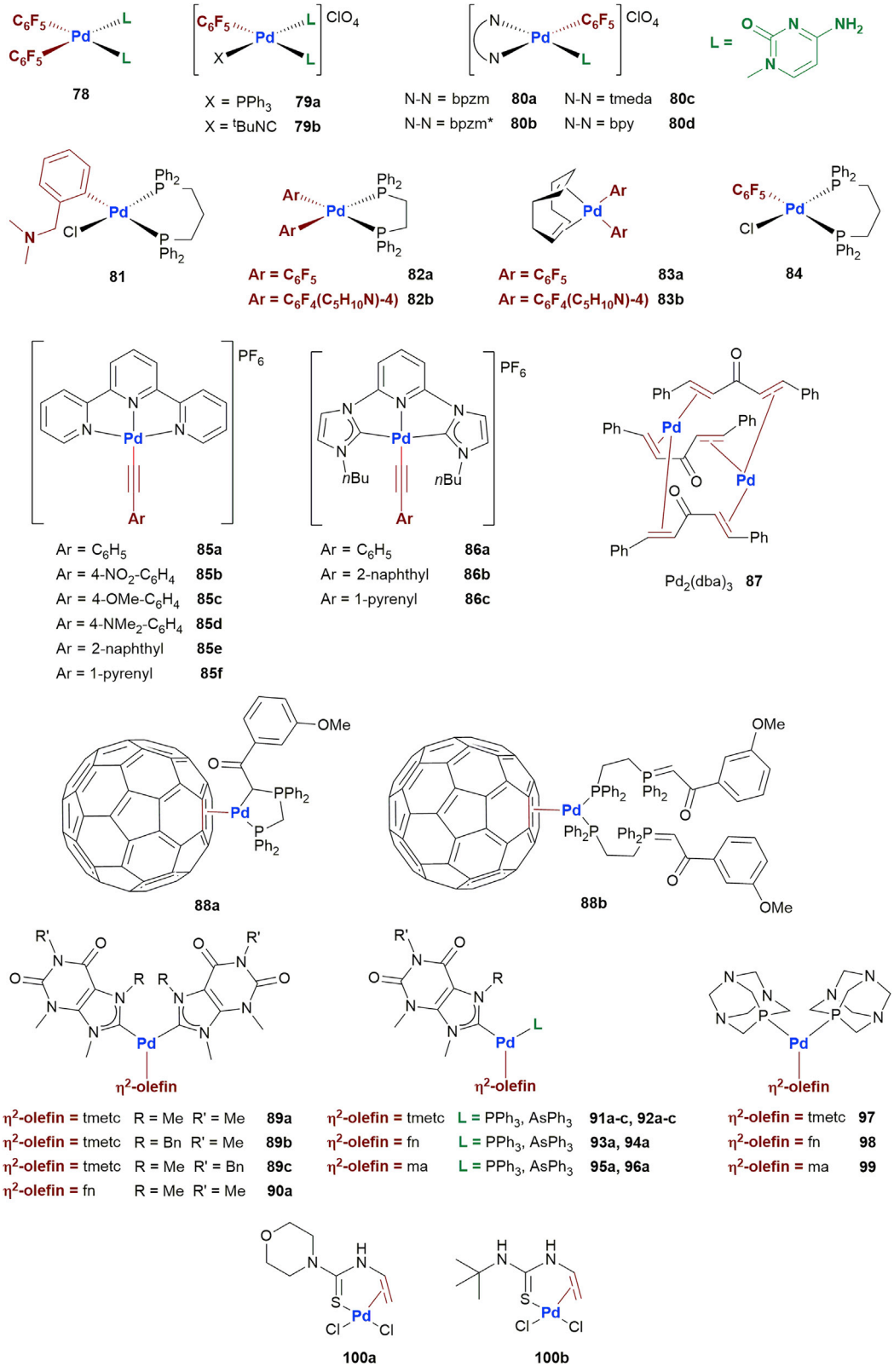

Figure 7. Aryl-, alkynyl-, and olefin-palladium complexes as potential anticancer agents

Chemical structure of the most promising Pd(II)-aryl, Pd(II)-alkynyl, and Pd(0/II)-olefin tested *in vitro*.

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of $Pd_2(dba)_3$ (dba, dibenzylideneacetone), the anticancer properties of Pd(0)-olefin complexes remained substantially unexplored until 2018. It should be remembered that $Pd_2(dba)_3$ (**87**), generally used as a precursor of Pd(0) and Pd(II) complexes, has been widely studied in the last 15 years by Arbiser and coworkers[92–94] as a potential drug against various neoplasms such as melanoma, chronic lymphocytic leukemia, and multiple myeloma. In addition to the numerous *in vitro* and *in vivo* experiments that attest to its effectiveness, interesting biological assays suggested that it acts without releasing dibenzylideneacetone molecules. This diolefin has its own antitumor activity but with completely different efficacy and mechanism of action.

The first study of the antitumor activity of Pd(0)-olefin complexes other than classical $Pd_2(dba)_3$ was reported by Sabounchei[95] in 2018. These authors described the synthesis and anticancer activity of novel Pd(0)-[60] fullerene complexes bearing phosphorus ylides with a different coordination mode (**88a–b**). Both compounds were found to be moderately active against KB cells ($IC_{50}^{(72h)}$ = 68 and 33 μM) and poorly active toward U87 and HeLa cells ($IC_{50}^{(72h)}$ > 150 μM). It is noteworthy that their platinum congeners also showed a certain specificity for the KB line.

A year later, Visentin and Scattolin[96] published the first systematic study of antitumor activity toward A2780 and A2780*cis* cell lines of Pd(0) complexes bearing different olefins (tmetc, tetramethyl-1,1,2,2-ethene tetracarboxylate; fn, fumaronitrile; and ma, maleic anhydride) and at least one purine-based *N*-heterocyclic carbene (**89–96**). Most of the tested compounds exibited significant activity toward both tumor cells, in many cases comparable to cisplatin ($IC_{50}^{(72h)}$ [**89–96** and cisplatin] = 0.8–10 and 0.6 μM [A2780], 1.1–51.3 and 5.6 μM [A2780*cis*]). Both antiproliferative and proapoptotic assays seem to suggest that complexes with fumaronitrile and maleic anhydride are usually more active than analogs with tetramethyl-1,1,2,2-ethene tetracarboxylate and that the best combination, regardless of the coordinated olefin, is represented by the contextual presence of NHC and triphenylphosphine ligands (**91a–c**, **93a**, and **95a**). Moreover, in the same report, some Pd(0)-olefin complexes coordinating 2 PTA ligands (**97–99**) were synthesized and tested. Among these derivatives, **98** is particularly promising since it combines a significant antiproliferative activity against ovarian cancer cells with a low cytotoxicity toward MRC-5 fibroblasts ($IC_{50}^{(72h)}$ = 2.9 μM [A2780], 5.4 μM [A2780*cis*], and 72 μM [MRC-5]).

In 2017, Repich and coworkers[97] described the cytotoxic, cytostatic, and proapoptotic activities of 2 interesting Pd(II)-olefin complexes (**100a–b**) toward HeLa cancer cells. Interestingly, **100a** was significantly less cytotoxic than **100b** ($IC_{50}^{(48h)}$ = 150 and 2 μM, respectively), but at a concentration of IC_{50}/10 (15 and 0.2 μM, respectively), the former exhibited more pronounced cytostatic and proapoptotic effects compared to **100b** and cisplatin. These results, which are comparable to those obtained with their platinum congeners, have been confirmed by DNA affinity studies carried out at different concentrations.

Palladium-allyl complexes

The last promising class of organopalladium complexes that we address here is represented by the Pd(II)-η^3-allyl derivatives. These compounds are well known as efficient homogeneous catalysts, especially in cross-coupling reactions,[98–100] and for their ability to transfer the allyl residue onto a target substrate by means of the well-known allylation process.[101–103] The first Pd(II)-η^3-allyl complex tested *in vitro* is attributed to Li and coworkers,[104] who demonstrated the good cytotoxicity of **101** (see Figure 8) toward MCF-7, MDA-MB-231, and U87 cancer cells ($IC_{50}^{(48h)}$ = 4.50–10.25 μM).

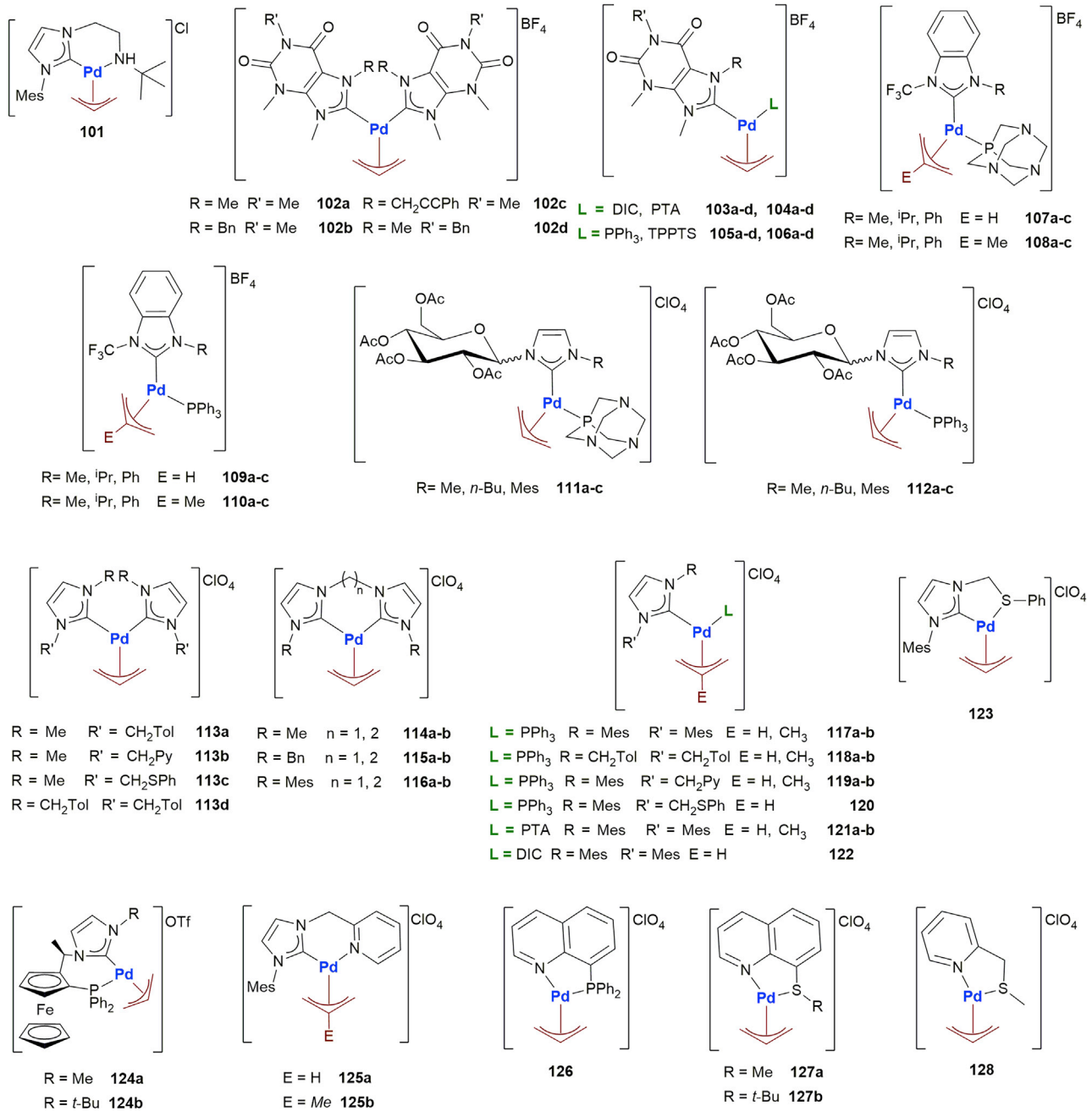

Figure 8. Palladium(II)-η^3-allyl complexes with promising anticancer properties

Categories of Pd(II)-η^3-allyl complexes investigated by the Li and Visentin groups toward cancer cell lines and tumoroids extracted from patients.

Inspired by these promising results, Visentin and Scattolin[105–108] recently reported the synthesis and antitumor activity of a wide range of Pd(II)-η^3-allyl complexes. In their initial work, they demonstrated the good cytotoxicity of Pd(II)-η^3-allyl complexes bearing at least one purine-based NHC ligand (**102–106**) toward A2780 and SKOV3 cancer cells.[105] In particular, complexes **104d**, **105c**, and **105d**, which bear PTA or PPh_3 as co-ligands, displayed similar or higher antiproliferative and pro-apoptotic activities than cisplatin ($IC_{50}^{(72h)}$ [**104d**, **105c–d**, and cisplatin] = 0.09–0.8 and 1.5 μM [A2780], 1.7–50.5 and 5.9 μM [SKOV3]). Moreover, the most active

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compounds against cancer cells were almost inactive toward normal ones ($IC_{50}^{(72h)}$ > 100 μM [MRC-5]).

In 2020, the same research group[106,107] focused on the category of mixed NHC/PR_3 (PR_3 = PPh_3 or PTA) Pd(II)-η^3-allyl complexes using *N*-trifluoromethyl (**107–110**) or carbohydrate-based (**111–112**) *N*-heterocyclic carbene ligands. All of the synthesized compounds exhibited potent antiproliferative activity against different cell lines, especially of ovarian cancer ($IC_{50}^{(96h)}$ = 0.02–0.76 μM [A2780], 0.22–1.4 μM [A2780*cis*], 0.3–10 μM [OVCAR5], 0.041–10 μM [A549], 2.8 μM [DLD-1], 0.21–14 μM [A375]). Curiously, the use of PPh_3 instead of PTA generally increases the cytotoxicity, but it also reduces the selectivity toward cancer cells. In this respect, the high cytotoxicity of complexes containing PPh_3 even toward normal cells seems to be compatible with a decomposition pathway that produces triphenylphosphinoxide. The latter presents an intrinsic toxicity that can damage cancer and healthy cells indiscriminately. Moreover, a reduction in the antiproliferative activity of approximately an order of magnitude was observed by introducing a methyl substituent on the 2-position of the allyl fragment (i.e., **107a** versus **108a**). These observations seem to suggest that the anticancer activity of these organometallic compounds may involve, as a key step, the nucleophilic attack on the allyl fragment by a specific nucleophilic site present in the biotarget. In addition, a series of immunofluorescence assays, aimed at defining the cellular targets of these palladium complexes, has shown that mitochondria are damaged before DNA, thus revealing a behavior substantially different from that of cisplatin.

Interestingly, the representative complex **108c** was also very active against ovarian cancer tumoroids derived from patients and showed a low toxicity toward normal liver organoids. This high activity and selectivity, which need to be confirmed by *in vivo* experiments, make this class of compounds particularly promising in the arsenal of palladium-based anticancer agents.

The versatility of Pd(II)-η^3-allyl complexes was illustrated by an extensive screening of different spectator monodentate and bidentate ligands (complexes **113–128**) that have highlighted the high antiproliferative activity against 6 different tumor lines for almost all of the tested compounds ($IC_{50}^{(96h)}$ = 0.019–0.37 μM [A2780], 0.033–4.8 μM [A2780*cis*], 0.28–2.9 μM [OVCAR5], 0.07–5 μM [A549], 0.26–9 μM [DLD-1], 0.022–11 μM [A375]).[108] This seems to confirm that the cytotoxicity of these species is mainly ascribable to the Pd-allyl fragment. In addition, since the less active compounds bear labile N-S ligands (**127–128**), we can say that the strength of the metal-spectator ligand bond is an important factor in promoting the cytotoxicity of Pd(II)-η^3-allyl derivatives. Finally, compounds **113a–c**, **115a–b**, **121a–b**, and **123** appear the most promising among those tested, as they combine high cytotoxicity against cancer cells with low toxicity toward normal ones ($IC_{50}^{(96h)}$ > 100 μM [MRC-5]).

Design of organopalladium anticancer agents: a structure/activity analysis

Based on the results presented in the previous chapters, here we offer an overview of the combinations of ligands that have proved to be particularly effective for obtaining organometallic palladium compounds with high *in vitro* anticancer activity. In particular, Table 1 shows the most active compounds among those recently synthesized, highlighting their structural properties, IC_{50} values toward cancer cells, mode of action, and/or additional information.

Although it is difficult to compare biological data obtained from different laboratories, which are often attributable to different cell lines, incubation times, and analysis methods, some general considerations on the structure/activity relationship of

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Table 1. An overview of the most promising organopalladium complexes

Category	Complex	Cancer cell lines (average IC_{50})	Mode of action and further details
Mononuclear cyclopalladates with benzophenone imines (N-H)	**1a–b**	4 μM (MCF-7), 1 μM (MDA-MB-231), 18–20 μM (HCT-116)	–
Tetranuclear palladacyclic complexes	**13a–c**	5.5–6.5 μM (HT-29), 5.8-7.0 μM (HepG2)	apoptosis death; intercalative binding with DNA; low toxicity toward normal cells
Dinuclear cyclopalladates bearing dppf, bpp, and dppe as bridging diphosphines	**14a–b**	2.3–5.7 μM (SKOV3), 5.2–6.7 μM (JURKAT)	intercalative binding with DNA; low toxicity toward normal cells
	15a–b	7.5 μM (MCF-7), 5.3–7.2 μM (HT-29), 7.5–8.5 μM (HeLa), 2.5–3.1 μM (K562)	
	16	6.5 μM (4C11-), 10.0 μM (Tm5)	apoptosis involving the lysosomal mitochondrial axis; high p62 protein level suggests a blocked autophagy
Mono- and dinuclear ferrocene cyclopalladated compounds	**17a–d**	1.3–3.2 μM (MCF-7), 6.4–9.2 μM (MDA-MB-231), 3.0–5.1 μM (HCT-116), 4.6–9.1 μM (HeLa)	–
	18a–d	0.4–0.9 μM (MCF-7), 4.0–8.0 μM (MDA-MB-231), 1.6–4.3 μM (HCT-116), 3.6–6.9 μM (HeLa)	–
	19–20	0.2–0.6 μM (KGN), 0.1–0.4 μM (4T1), 0.5–0.7 μM (HepG2)	–
Mononuclear cyclopalladates with O^O ligands	**22a–b**	2.8–5.8 μM (MCF-7)	strong affinity with DNA (via intercalation) and BSA (via Sudlow's site 1)
Mixed NHC/L (L = PPh_3, PTA, DIC) and bisNHC palladacyclopentadienyl complexes	**33–34**	0.6–4.3 μM (A2780), 0.6–2.1 μM (A2780*cis*)	apoptosis; comparable activity between CisPt sensitive and CisPt resistant cell lines; low toxicity toward normal cells
	35–38	0.04–3.9 μM (A2780), 0.6–12 μM (A2780*cis*), 0.13–13 μM (OVCAR5), 0.2–17 μM for **37–38** (A549), 0.1 μM for **36a** (DLD-1), 0.3-29 μM (A375)	**35–36** are stable in the presence of GSH; **35a** acts primarily on DNA; low toxicity toward normal cells
Zwitterionic palladacycle with a C^N 10-membered coordinative ring	**43b**	0.51 and 1.12 μM (A2780, A2780*cis*), 4.4 μM (OVCAR5), 7.0 and 4.6 μM (SKOV3, SKOV3*cis*), 3.5 μM (A549), 4.4 μM (DLD-1), 0.3 μM (A375)	low toxicity toward normal cells
Palladacycles with a P(V) phosphine-sulfide arm	**44f–h**	4–13 μM (MCF-7), 2.5–7.5 μM (HCT-116)	–
Palladacycles with polypyridyl ligands	**45a**	0.9 μM (light), 12 μM (dark) (A549) 5.0 μM (light), 20 μM (dark) (A431)	enhanced cytotoxicity under blue light activation
	46a	0.33 μM (light), 2.2 μM (dark) (A549) 4.8 μM (light), 45 μM (dark) (A431)	enhanced cytotoxicity under blue light activation; studies on mice model and 3D tumoroids

(*Continued on next page*)

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Table 1. *Continued*

Category	Complex	Cancer cell lines (average IC_{50})	Mode of action and further details
C^N^N palladacyclic complexes bearing NHC ligands	**47a–d**	0.2–1.7 μM (A2780), 0.5–2.2 μM (A2780*cis*), 0.5–0.9 μM (MDA-MB-231), 0.09–2.5 μM (NCI-H1650), 0.08–2.1 μM (NCI-H460), 0.1–1.8 μM (HeLa)	Potent *in vitro* and *in vivo* anticancer activity; mitochondrial dysfunction, antiangiogenic activity and inhibition of EGFR
Macrocyclic Pd complexes with tetra-NHC ligands	**52a–b**	0.1–1.9 μM (A2780*cis*), 0.4–2.8 μM (HeLa)	–
Pd(I) dimer bearing chelating bisNHC ligands	**53**	0.025 and 1.9 μM (A2780, A2780*cis*) 1.4 and 3.0 μM (OVCAR5, OVCAR3), 0.38 μM (KURAMOCHI), 0.38 μM (A549), 2.8 μM (DLD-1), 3.5 μM (HeLa)	potent *in vitro* and *ex vivo* anticancer activity; low toxicity toward normal cells; mitochondrial dysfunction
trans-$[Pd(NHC)_2Cl_2]$ with benzyl and *t*-butyl substituents	**54a**	1 μM (MCF-7), 0.8 μM (HCT-116), 4 μM (HeLa)	cell-cycle arrest in G2 phase leading to p-53-dependent apoptosis
cis-$[Pd(NHC)_2Br_2]$ and *cis*-$[Pd(NHC)(MeCN)Cl_2]$	**61–62**	1.3–5.8 μM (HCT-116)	–
trans-$[Pd(NHC)_2(OTf)_2]$ bearing triazole-based NHCs	**65d**	0.55–0.7 μM (MCF-7)	cell-cycle arrest in G2 phase leading to p-53-dependent apoptosis
trans-$[Pd(NHC)(Py)Cl_2]$ and *trans*-$[Pd(NHC)(PPh_3)Cl_2]$ with 2-methoxyehtyl substituents	**74a–i**	0.52–1.9 μM (MCF-7), 0.71–1.5 μM (MDA-MB-231)	–
	75a–h	0.6–1.9 μM (MCF-7), 0.4–1.55 μM (MDA-MB-231)	–
Palladium aryl complexes (aryl = C_6F_5)	**78–80**	4.7–11.3 μM (HL-60)	apoptosis; modification of the pBR322 DNA similar to cisplatin (**78**)
	84	0.6–0.9 μM (L1210 and L1210/DDP)	–
[Pd(C^N^C)]-alkynyl complex	**86c**	0.2 μM (light), 1.5–2.8 μM (dark) (MDA-MB-231)	enhanced cytotoxicity under visible light activation; ROS production; low affinity with DNA
Mixed NHC/PPh_3 and bisPTA Pd(0)-η^2-olefin complexes	**91b–c**, **93a** and **95a**	0.8–5.4 μM (A2780), 4.8–6.1 μM (A2780*cis*)	Apoptosis; low toxicity toward normal cells
	98	2.9 μM (A2780), 5.4 μM (A2780*cis*)	Apoptosis; low toxicity toward normal cells
Pd(II)-η^2-olefin complex	**100b**	2 μM (HeLa)	–
Pd(II)-η^3-allyl complex bearing a bidentate NHC-amine ligand	**101**	6.47 μM (MCF-7), 4.50 μM (MDA-MB-231) 10.25 μM (U87)	–
Mixed xanthine-based NHC/L (L = PPh_3, PTA) Pd(II)-η^3-allyls	**104d**, **105c**, and **105d**	0.09–0.8 μM (A2780), 1.7–50.5 μM (SKOV3)	apoptosis; low toxicity toward normal cells
Mixed NHC/PTA (CF_3-benzimidazole and carbohydrate-based NHCs) Pd(II)-η^3-allyls	**107a–c and 108a–c**	0.02–0.76 and 0.22–1.4 μM (A2780, A2780*cis*), 0.3–10 μM (OVCAR5), 0.21–10 μM (A549), 2.8 μM (DLD-1), 0.21–14 μM (A375)	potent *in vitro* and *ex vivo* anticancer activity; low toxicity toward normal cells; mitochondrial dysfunction
	111a–c	0.28–0.48 and 0.30–1.0 μM (A2780, A2780*cis*), 1.7–9 μM (OVCAR5), 0.041–0.295 μM (A549), 4.5–13 μM (A375)	potent *in vitro* and *ex vivo* anticancer activity; low toxicity toward normal cells

(*Continued on next page*)

Table 1. *Continued*

Category	Complex	Cancer cell lines (average IC_{50})	Mode of action and further details
bisNHC (with picolyl and thioether groups) **113b–c**, chelating bisNHC (with benzyl groups) **115a–b**, mixed NHC/PTA **121a–b** and chelating NHC-thioether **123** Pd(II)-η^3-allyls	**113b–c**, **115a–b**, and **121a–b** and **123**	0.019–0.37 and 0.033–4.8 μM (A2780, A2780*cis*), 0.28–2.9 μM (OVCAR5), 0.07–5 μM (A549), 0.26–9 μM (DLD-1), 0.022–11 μM (A375)	potent *in vitro* anticancer activity; low toxicity toward normal cells

organopalladium complexes can be made. It appears clear from that most of the investigated cationic complexes (e.g., **47a–d**, **53**, **86c**, **107a–c**, and **108a–c**) seem to induce apoptosis death due to important alterations of the mitochondrial membrane, with an evident impact on the cellular respiratory chain. On the contrary, most of the neutral complexes (e.g., **13a–c**, **14a–b**, **15a–b**, **22a–b**, **33a**, and **78**) seem to target primarily DNA, acting as structural compounds and promoting an intercalative binding. This behavior is mainly due to the presence of spectator ligands strongly anchored to the metal center.

Being the metal center in oxidation state +2 in all of the complexes whose mechanism of action has been investigated (with the sole exception of Pd(I) dimer **53**), it is not possible to define a correlation between mode of action and oxidation state. However, the fact that Pd(II) compounds exhibit different biotargets (mainly based on the charge of the complex) seems to suggest that the oxidation state of the metal is not a decisive factor in the mechanism of action of the complexes.

The different mode of action exhibited by most of the investigated palladium organometallic complexes with respect to cisplatin and its second- and third-generation derivatives (carboplatin and oxaliplatin), which are still the reference metallodrugs in cancer therapy, is proved by their cytotoxicity toward cisplatin-resistant cancer cells. For what concerns the influence of supporting ligands on antitumor activity, it is possible to observe that, within the broad family of Pd-η^3-allyl complexes, those coordinating one NHC ligand (NHC = classical imidazolylidenes, trifluoromethyl benzimidazolylidenes, xanthine-based NHCs, or carbohydrate-based NHCs) and one PTA molecule are particularly promising (**104d**, **107a–c**, **108a–c**, and **121a–b**). As a matter of fact, this ligand combination generally ensures a good antiproliferative activity toward cancer cells and, at the same time, a poor cytotoxicity toward normal ones. Similarly, the best ligand combination for Pd(0)-olefin complexes, regardless of the olefin used, is represented by one NHC and one PPh_3 ligand (**91b–c**, **93a**, and **95a**). As for the aryl complexes, only those containing the pentafluorophenyl fragment (**78–80**) exhibited remarkable anticancer activity.

Within the category of acyclic palladium complexes bearing NHC ligands, the most active compounds against breast cancer lines (**54a**, **61–62**, **65d**, **74a–l**, and **75a–h**) do not follow a general trend of structure/activity relationship. The only exceptions are the *trans*-[Pd(NHC)(Py)Cl_2] and *trans*-[Pd(NHC)(PPh_3)Cl_2] complexes, in which the presence of 2-methoxyehtyl substituents in the carbene moiety seems to play a key role.

Finally, among the palladacyclic derivatives, **47a–d** and **33–38**, coordinating at least one NHC ligand, are particularly promising. This design ensures high stability in solution, even in the presence of biological thiols (e.g., reduced glutathione, GSH), potent *in vitro* anticancer activity, and moderate toxicity toward non-cancerous cells.

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In the panorama of the compounds reported in Table 1, those that exhibited excellent *in vitro* anticancer activity (IC_{50} values in the sub-micromolar range) were also investigated in more complex biological systems such as mice model and/or 3D tumoroids/organoids. As for the mice model, complexes **46a** and **47d** proved to be particularly effective in significantly reducing the tumor tissue and, at the same time, showing good values of maximum tolerated dose.

Very interesting results were obtained for compounds **46a**, **53**, and **108c** using 3D organoids. Organoids are lab-built mini-organs that can act as models to recapitulate cancer development. The availability of innovative biobanks of tumoroids/organoids represents a revolutionary tool to study protein function in the onset and development of cancer or to develop innovative therapies.[106]

The investigated compounds showed high cytotoxicity toward tumoroids derived from patients, especially those affected by ovarian cancer, and low cytotoxicity toward normal liver organoids.

Summary and outlook

We believe that this overview of organopalladium complexes may offer numerous stimuli and insights for researchers in the field of metallodrugs. The high *in vitro* and *ex vivo* anticancer activity exhibited by some categories of palladium complexes, even toward tumors resistant to cisplatin and its derivatives, combined with often different modes of action than platinum-based compounds is the key to their growing success. A confirmation of the real potential of the compounds that have shown potent *in vitro* anticancer activity is expected soon based mainly on *in vivo* tests. Further developments may come from the study of novel organometallic fragments or fragments whose reactivity and/or catalytic activity is known but whose biological activity is unexplored. It should be remembered that, despite the presence of new and innovative therapeutic protocols for cancer therapy, the development of new, efficient, and selective anticancer agents is a still crucial field of study. Every step forward offered by the scientific community to this field increases hope in the challenge against what remains one of the most lethal pathologies of the 21st century.

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AUTHOR CONTRIBUTIONS

Supervision, F.V. and S.P.N.; conceptualization, T.S., V.A.V., F.V., and S.P.N.; visualization, T.S., V.A.V., F.V., and S.P.N.; writing – original draft, T.S. and V.A.V.; writing – review & editing, T.S., V.A.V., F.V., and S.P.N.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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