



Anticancer activity of palladium-based complexes against triple-negative breast cancer

Martin Vojtek¹, Maria P.M. Marques^{2,3}, Isabel M.P.L.V.O. Ferreira⁴,
Hélder Mota-Filipe⁵ and Carmen Diniz¹



¹ LAQV/REQUIMTE, Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal

² Unidade de I&D “Química-Física Molecular”, Department of Chemistry, University of Coimbra, Coimbra, Portugal

³ Department of Life Sciences, University of Coimbra, Coimbra, Portugal

⁴ LAQV/REQUIMTE, Laboratory of Bromatology and Hydrology, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal

⁵ iMed.Ulisboa, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal

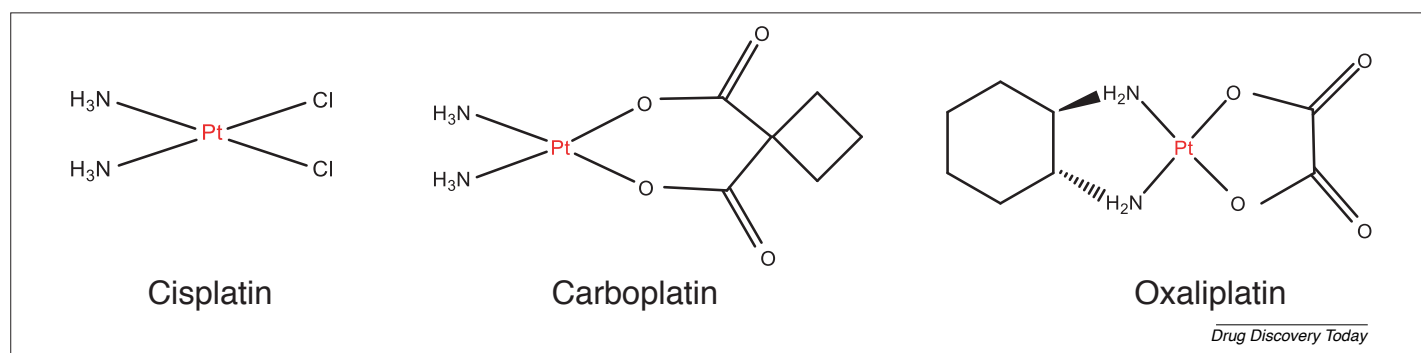
Treatment of triple-negative breast carcinoma (TNBC) remains an unmet medical need with no targeted therapy available to date. Accounting for 10–30% of all human breast cancer tumors, this mammary carcinoma subtype has a particularly poor prognosis owing to its high metastatic potential, aggressive biology and limited pharmacological treatment options. Platinum chemotherapeutics are the mainstay therapy in patients with TNBC but their clinical use is limited by severe toxicity and acquired resistance. Palladium-based complexes are appealing alternative metal-based drugs because of significant similarities regarding structure and coordination chemistry with the platinum agents. This review summarizes the knowledge gathered so far on 121 Pd(II) complexes, emphasizing their anticancer activity and putative pharmacological targets toward TNBC.

Introduction

Breast cancer is the most common type of cancer in women worldwide [1]. By 2030, the worldwide burden of new breast cancer cases is expected to increase by 50%, from 2.1 million new cases/year in 2018 to 3.2 million/year in 2030 [2,3]. Despite the noteworthy advances in breast cancer treatment over the past decades, survival for the triple-negative breast carcinoma (TNBC) subtype, which comprises 10–30% of all breast cancer tumors [4], remains poor owing to its high metastatic capacity and limited pharmacological treatment options. Currently, no specific targeted approach is available for TNBC and chemotherapy remains the only pharmacological treatment option [5]. The serendipitous discovery of the platinum(II) [Pt(II)] agent cisplatin, in the 1970s, commenced a novel era for metal-based drugs [6,7], and to date cisplatin is in use for the treatment of a variety of malignancies, including breast, testicular, ovarian, cervical, prostate, head and neck, bladder and lung cancers, as well as refractory non-

Hodgkin's lymphoma [5]. The pharmacological action of cisplatin is mediated by DNA binding, interfering with transcription, DNA replication and inducing apoptosis [8,9]. Despite its clinical success, several drawbacks such as acquired resistance and severe side effects (nephro-, hepato-, neuro- and oto-toxicity in combination with bone marrow suppression) have directed substantial efforts to develop other Pt(II) analogs and novel metal-containing drugs [5,10,11]. Furthermore, the main concern regarding platinum-based therapy is the development of resistance over the course of treatment. Although the mechanism(s) responsible for these effects have not been fully elucidated, processes such as changes in drug uptake or efflux, increased activity of the DNA repair system and inhibition of apoptosis are known to be involved [8,10]. Despite the numerous metal-based molecules developed over the past years, only two other platinum agents – carboplatin and oxaliplatin – have received worldwide approval for clinical practice so far [12] (Fig. 1). Thus, the main goal in metal-based drug discovery is to develop compounds with improved therapeutic efficacy, reduced toxicity and lack (or minimal) of acquired

Corresponding author: Diniz, C. (cdiniz@ff.up.pt)

**FIGURE 1**

Structures of the platinum agents approved for clinical use worldwide.

resistance as compared with clinically used Pt(II) compounds [13]. Accordingly, some palladium(II) [Pd(II)] complexes have already shown promising antitumor activity against a variety of human cancers, often surpassing cisplatin and other platinum drugs [14], particularly against TNBC as discussed further.

TNBC heterogeneity

Breast cancer is a heterogeneous disease with distinct subtypes that differentially respond to pharmacological therapy with variable rates of failure and mortality. The combined immunohistochemistry evaluation of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER)2 overexpression stratifies all breast cancer tumors, based on the receptor status, in ER-positive (ER⁺/HER2⁻), HER2-positive (ER⁻/HER2⁺), triple-positive (ER⁺/PR⁺/HER2⁺) and triple-negative (ER⁻/PR⁻/HER2⁻) breast cancer subtypes [15–17]. The patients with ER⁺ and/or HER2⁺ subtype tumors can be treated with targeted therapies, only effective in these subsets of patients. However, for patients with TNBC tumors (ER⁻/PR⁻/HER2⁻) there is still no specific targeted therapy available. Likewise, a high rate of distant recurrences contributes to the fact that <30% of women with metastatic TNBC survive for 5 years, and almost all of them die owing to the disease despite adjuvant (post-surgical) chemotherapy [18].

In the past two decades, at least five intrinsic breast cancer subtypes (luminal A, luminal B, HER2-enriched, basal-like and normal-like breast cancer) have been identified based on distinct genetic signatures and substantial differences in incidence, survival and response to treatment [19,20]. In 2011, the approximation of intrinsic subtypes to immunohistochemistry classification was proposed along with the recommendations for systemic treatment for each of the subtypes: endocrine therapy alone for luminal A; endocrine ± chemotherapy for luminal B (HER2⁻); chemotherapy + anti-HER2 + endocrine therapy for luminal B (HER2⁺); chemotherapy + anti-HER2 for HER2⁺ (non-luminal) and chemotherapy for TNBC [21].

Over the past years, basal-like breast cancer has become the synonym for TNBC because TNBC tends to have a basal-like breast cancer pathology. However, not all TNBCs fall into the basal-like intrinsic subtype, and not all basal-like cancers are TNBC, having 20–30% discordance between the two definitions. Therefore, significant heterogeneity exists among the patients diagnosed with TNBC, which suggests that the final molecular taxonomy of breast cancers

is likely to be even more complex [17,22]. As previously demonstrated, the majority of TNBC tumors fall into basal-like (50–75%) [23] and claudin-low (25–39%) subtypes, whereas all the other non-basal subtypes include HER2-enriched (7–14%), luminal B (4–7%), luminal A (4–5%) and normal-breast-like (1%), depending on the population [24,25]. Another approach to TNBC heterogeneity has been proposed by Lehmann and colleagues that identified seven distinct molecular subtypes of TNBC: two basal-like subtypes, immunomodulatory, mesenchymal, mesenchymal-stem-like, luminal androgen receptor and normal-like subtypes [26].

Advances in TNBC targeted therapies

The treatment of patients with TNBC remains the biggest challenge of breast cancer therapy without generally accepted guidelines on chemotherapy backbone for the neoadjuvant, adjuvant and metastatic settings [27,28]. The chemotherapy drugs generally used are anthracyclines, taxanes and platinum-based agents [29,30]. Anthracyclines (e.g., doxorubicin, epirubicin, mitoxantrone) are the most effective anticancer drugs ever developed, preventing DNA replication and transcription [31]. Taxanes (e.g., docetaxel, paclitaxel) are diterpenes that inhibit cell division by stabilization of microtubules and prevent their depolymerization, thus blocking cells in the G2/M phase of the cell cycle and inducing apoptosis [32]. Platinum agents (e.g., cisplatin, carboplatin, oxaliplatin) are non-cell-cycle-specific metal-based drugs targeting DNA to form inter- and intra-strand short-range cross-links that affect DNA replication and induce apoptosis [8]. The use of platinum coordination complexes is particularly favorable in the treatment of TNBC tumors with the breast cancer susceptibility gene (BRCA)1 mutation [29].

The profound understanding of molecular differences in biology of TNBC and non-TNBC is the fundamental factor for a successful targeted therapy. At present, numerous molecular-oriented studies have identified different putative targets specific to TNBC, providing a strong rationale for the design and development of targeted therapeutic approaches. Emerging discoveries of molecular alterations in TNBC tumors (pathway activations, mutations or receptor enrichments) has sparked interest in targeting the p53 and BRCA1/2 mutations, poly(adenosine diphosphate-ribose) polymerase (PARP) enzymes, epidermal growth factor receptor (EGFR), androgen receptor (AR), serine/threonine kinase/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway and vascular

endothelial growth factor (VEGF) with approved drugs rather than with new molecules.

Inhibition of VEGF and its receptor

VEGF is overexpressed in >30% of TNBC cases and has a crucial role in proliferation, invasion and metastasis owing to its key role in promoting angiogenesis. This fact supported clinical trials for incorporating an antiangiogenic treatment in TNBC patients. In metastatic settings, the use of bevacizumab improved the patient progression-free survival, whereas the overall survival remained unchanged. Additionally, the inhibition of VEGF receptors with tyrosine kinase inhibitors (such as sunitinib or sorafenib) was also investigated in clinical trials that yielded inconclusive data on the progression-free survival and the appearance of adverse events [29,33].

PARP inhibitors

PARP enzymes play a major part in DNA repair mechanisms. Particularly, the most abundant PARP1 isoform has a key role in ssDNA breaks. Inhibition of PARP activity leads to the accumulation of ssDNA breaks, which are normally repaired by double-strand homologous recombination pathways that include tumor-suppressor proteins BRCA1 and BRCA2. Thus, TNBC with BRCA1/2 germline mutations as well as the BRCAness phenotype are particularly vulnerable for targeting the PARP-mediated DNA repair mechanism [33]. Olaparib, one of the PARP inhibitors used for ovarian cancer treatment, has already been approved by the FDA (January 2018) for the treatment of BRCA-mutated HER2[−] breast cancer and is awaiting approval from the European Medicines Agency (EMA). Furthermore, other PARP inhibitors including veliparib and iniparib have been developed and studied in clinical trials [33].

Inhibition of EGFR

The overexpression of EGFR is reported in 50% of TNBC patients, providing rationale for its targeting with monoclonal antibodies (e.g., cetuximab). Some clinical trials reported an encouraging increase of progression-free survival, even though these trials enrolled a small number of patients. However, it is important to determine the subpopulation of TNBC patients that would benefit from EGFR inhibition [29].

Targeting the p53 mutation

The majority of TNBC tumors (80% of basal TNBC tumors) carry a mutation in the *TP53* gene, which encodes the tumor-suppressor protein p53 required for G1 checkpoint regulation in the presence of genotoxic stress [34]. Thus, targeting p53 has attracted great attention and this is supported with encouraging preclinical *in vitro* data [34,35]. However, to date, the clinical trials in TNBC have not established the efficacy of targeting p53 in the clinic.

PI3K/AKT/mTOR pathway inhibition

The PI3K/AKT pathway is hyperactivated in ~10% of TNBC patients, providing a rationale for its targeting. mTOR promotes protein translation, angiogenesis, proliferation, migration and metabolism. The mTOR inhibitor everolimus has already proved its clinical benefits for the treatment of postmenopausal women with advanced hormone-receptor-positive/HER2[−] breast cancer.

However, its use in TNBC patients is still being studied in clinical trials to establish suitable combination regimens [27,33].

AR inhibition

Approximately 10% of TNBC has been classified as Luminal Androgen Receptor (LAR) subtype, overexpressing the AR. The anti-androgen nonsteroidal inhibitor bicalutamide, used for the treatment of advanced prostate cancer, is being tested in several TNBC clinical trials, awaiting conclusive results and the establishment of treatment regimens [29].

Other emerging targets for TNBC therapy

Bromodomain and extraterminal inhibitors are a recently developed novel class of agents with demonstrated *in vitro* and *in vivo* effects to inhibit growth of several tumors, including TNBC. These inhibitors displace bromodomain and extraterminal proteins such as BRD4 from chromatin by competing with their acetyl-lysine recognition modules, leading to inhibition of oncogenic transcriptional programs [36,37].

Another emerging approach is targeting overexpressed extracellular signal regulated kinase (ERK)5 and its upstream activator MEK5 in TNBC. ERK5 belongs to the mitogen-activated protein kinase (MAPK) family of signal transducers, and its deregulation has been linked to breast cancer. Targeting MEK5/ERK5 with specific inhibitors has already shown promising results *in vitro* and *in vivo*, inducing apoptotic cell death in TNBC [38,39].

Palladium complexes against TNBC

Coordination complexes with transition metals are particularly appealing for medicinal chemistry owing to the variable oxidation states of the ion, coordination numbers and an ability to bind to a variety of ligands (O, S, N, P, C and halides). A key feature for the design of these transition-metal-based complexes is to find the optimal combination of *in vivo* thermodynamic and kinetic stabilities, strongly dependent on the nature of the ligand(s) and the leaving group(s) [40]. Presently, several metal-based compounds are under study, displaying promising antiproliferative and cytotoxic effects toward a wide range of human tumors. These include palladium(II) [41], gallium(III), ruthenium(II) [42] and ruthenium(III) [43], gold(I) and gold(III), bismuth(III), copper(II), molybdenum(II) [44] and tin(IV) [45–47] complexes.

The development of palladium coordination compounds is particularly challenging owing to their high lability and ~10⁵ faster ligand exchange rate than platinum analogs. Furthermore, relatively low solubility of the palladium-based complexes is another hurdle that limits their further course toward clinical trials. Notwithstanding these challenges, >800 anticancer Pd(II) complexes have already been discovered since the 1980s [14] and, in 2017, padeliporfin (TOOKAD[®]) was the first Pd(II) compound approved for clinical use. This Pd(II)-substituted bacteriochlorophyll derivative, used in photodynamic therapy in patients with prostate cancer [48], demonstrates the high potential of palladium agents for clinical applications, especially against chemotherapy-resistant cancers.

To the best of our knowledge, the present review is the first one focusing on the activity of palladium complexes exclusively in TNBC. We have reviewed studies performed between 1992 and 2018 that report on a total 121 palladium complexes (Table 1). The complexes were divided into eight groups according to structural

TABLE 1

Palladium complexes and their activity against triple-negative breast carcinoma

Group	Complex no.	Complex designation ^a	Cell line	IC ₅₀	Target/mode of action	Selectivity toward TNBC	Year Refs
Derivatives of ethyl diamine	1	1a <i>cis</i> -[Pd(H ₂ dasa)Cl ₂] 1b <i>cis</i> -[Pd(Et ₂ dasa)Cl ₂]	MDA-MB-468	72 h: 1a 4.61 μM 1b 4.72 μM Ref. drug: cisplatin 2.67 μM	Modification of secondary structure in isolated DNA	NA	1994 [60]
	2	2a dichlorido-(O,O'-diethyl-(S,S)-ethylenediamine-N,N'-di-(2,2'-di(4-hydroxy-benzyl))-acetate)-palladium(II) 2b dichlorido-(O,O'-dipropyl-(S,S)-ethylenediamine-N,N'-di-(2,2'-di(4-hydroxy-benzyl))-acetate)-palladium(II) 2c ichlorido-(O,O'-dibutyl-(S,S)-ethylenediamine-N,N'-di-(2,2'-di(4-hydroxy-benzyl))-acetate)-palladium(II) 2d dichlorido-(O,O'-dipentyl-(S,S)-ethylenediamine-N,N'-di-(2,2'-di(4-hydroxy-benzyl))-acetate)-palladium(II)	MDA-MB-231	48 h: 2a 135 μM 2b 72 μM 2c 36 μM 2d 18 μM	NA	NA	2014 [61]
	3	3a dichlorido[O,O'-diethyl-(S,S)-ethylenediamine-N,N'-di-2-(4-methyl)pentaneate]palladium(II) 3b dichlorido[O,O'-di-n-propyl-(S,S)-ethylenediamine-N,N'-di-2-(4-methyl)pentaneate]palladium(II) 3c dichlorido[O,O'-di-n-butyl-(S,S)-ethylenediamine-N,N'-di-2-(4-methyl)pentaneate]palladium(II) 3d dichlorido[O,O'-di-n-pentyl-(S,S)-ethylenediamine-N,N'-di-2-(4-methyl)pentaneate]palladium(II)	MDA-MB-453	72 h: 3a >200 μM 3b 93.04 μM 3c 35.31 μM 3d 48.43 μM Ref. drug: cisplatin 3.75 μM	DNA fragmentation, induction of apoptosis and sub-G1 cell cycle arrest	NA	2014 [62]
Derivatives of biogenic polyamines	4	4a Pd ₃ Spd ₂ Cl ₁₂ 4b Pd ₃ Spd ₂ Cl ₈ 4c Pd ₃ Spd ₂ Cl ₆	MDA-MB-468	24 h: 4a 9 μM 4b 1.25 μM 4c 0.9 μM Ref. drug: cisplatin 2.5 μM	Modification of secondary structure in isolated DNA	NA	1992 [63]
	5	5a PdPutCl ₄ 5b Pd ₂ Put ₂ Cl ₄ 5c PdPutCl ₂ + Pd ₂ Put ₂ Cl ₄ 5d Pd ₃ SpmCl ₆ 5e Pd ₂ SpmCl ₄	MDA-MB-468	5a 1.60 μM 5b 0.70 μM 5c 0.53 μM 5d 11.51 μM 5e 8.44 μM Ref. drug: cisplatin 2.50 μM	Modification of secondary structure in isolated DNA	NA	1993 [64]

TABLE 1 (Continued)

Group	Complex no.	Complex designation ^a	Cell line	IC ₅₀	Target/mode of action	Selectivity toward TNBC	Year Refs
Derivatives of benzyl amine/imine	5e	Pd ₂ SpmCl ₄	MDA-MB-231	72 h: 2.8 μM Ref. drug: cisplatin 3.2 μM	Induction of double-stranded DNA breaks (stronger effect than for cisplatin). Interference with microtubules. Synergism with cisplatin	Tested vs normal human fibroblasts (BJ)	2011 [65]
	6	Pd ₂ BENSpm	L56Br-C1	72 h: 0.4 μM	DNA damage. Reduction of GSH and polyamine levels	Tested vs normal breast epithelial cells (MCF-10A)	2013 [66]
	7	Pd ₃ NSpd ₂	L56Br-C1	Exposure to 100 μM for 72 h significantly reduced cell number	Reduction of ornithine decarboxylase activity. Prolongation of S phase length	Tested vs normal breast epithelial cells (MCF-10A)	2013 [67]
	8	8a [Pd(4-ClC ₆ H ₄ N=C(COC ₆ H ₅)C ₆ H ₄)OAc] ₂ 8b [Pd(4-ClC ₆ H ₄ N=C(COC ₆ H ₅)C ₆ H ₄)Cl] ₂	MDA-MB-468	8a 6.75 μM 8b 8.95 μM Ref. drug: cisplatin 2.67 μM	NA	NA	1996 [68]
	9	9a [Pd(C,N)-C ₆ H ₄ CH ₂ NH(Et)Cl(Py)] 9b [Pd(C,N)-C ₆ H ₄ CH ₂ NH(t-Bu)Cl(PPh ₃)] 9c [Pd ₂ (C,N-dmba) ₂ (μ-dppe)(Cl) ₂]	MDA-MB-468	72 h: 9a 2.4 μM 9b 3.3 μM 9c 2.3 μM Ref. drug: cisplatin 4.8 μM	NA	NA	2012 [69]
	10	10a [Pd {C ₆ H ₄ CPh = NH}(μ-OAc)] ₂ 10b [Pd{C ₆ H ₄ CPh = NH}(μ-Cl)] ₂ 10c <i>trans-N,P</i> -[Pd{C ₆ H ₄ CPh = NH}(OAc)(PPh ₃)] 10d <i>trans-N,P</i> -[Pd{C ₆ H ₄ CPh = NH}(Cl)(PPh ₃)]	MDA-MB-231	72 h: 10a 15 μM 10b 13 μM 10c 1.1 μM 10d 1.1 μM Ref. drug: cisplatin 6.5 μM	Modification of secondary structure in isolated DNA	NA	2013 [70]
	11	Includes 12 complexes 11a-l whose structures are given in	MDA-MB-231	72 h: 11a ~100 μM 11b ~100 μM 11c 4.6 μM 11d 13 μM 11e 40 μM 11f 32 μM 11g 5.2 μM 11h 2.7 μM 11i 25 μM 11j 17 μM 11k 1.4 μM 11l 1.0 μM Ref. drug: cisplatin 6.5 μM	Modification of secondary structure in isolated DNA	NA	2014 [71]

TABLE 1 (Continued)

Group	Complex no.	Complex designation ^a	Cell line	IC ₅₀	Target/mode of action	Selectivity toward TNBC	Year Refs
	12	12a Pd{C ₆ H ₄ (Ph)C=N-phenyl} ₂ (μ-OAc) ₂ 12b Pd{C ₆ H ₄ (Ph)C=N-1-naphtyl} ₂ (μ-OAc) ₂ 12c Pd{C ₆ H ₄ (Ph)C=N-benzyl} ₂ (μ-OAc) ₂ 12d Pd{C ₆ H ₄ (Ph)C=N-α-methylbenzyl} ₂ (μ-OAc) ₂ 12e Pd{C ₆ H ₄ (Ph)C=NH} ₂ (μ-OAc) ₂ 12f Pd{C ₆ H ₄ (Ph)C=N-phenyl} ₂ (μ-Cl) ₂ 12g Pd{C ₆ H ₄ (Ph)C=N-1-naphtyl} ₂ (μ-Cl) ₂ 12h Pd{C ₆ H ₄ (Ph)C=N-benzyl} ₂ (μ-Cl) ₂ 12i Pd{C ₆ H ₄ (Ph)C=N-α-methylbenzyl} ₂ (μ-Cl) ₂ 12j Pd{C ₆ H ₄ (Ph)C=NH} ₂ (μ-Cl) ₂ 12k <i>trans</i> -N,P-[Pd{C ₆ H ₄ (Ph)C=N-phenyl}OAc(PPh ₃)] 12l <i>trans</i> -N,P-[Pd{C ₆ H ₄ (Ph)C=N-1-naphtyl}OAc(PPh ₃)] 12m <i>trans</i> -N,P-[Pd{C ₆ H ₄ (Ph)C=N-benzyl}OAc(PPh ₃)] 12n <i>trans</i> -N,P-[Pd{C ₆ H ₄ (Ph)C=N-α-methylbenzyl}OAc(PPh ₃)] 12o <i>trans</i> -N,P-[Pd{C ₆ H ₄ (Ph)C=NH}OAc(PPh ₃)] 12p <i>trans</i> -N,P-[Pd{C ₆ H ₄ (Ph)C=N-phenyl}Cl(PPh ₃)] 12q <i>trans</i> -N,P-[Pd{C ₆ H ₄ (Ph)C=N-1-naphtyl}Cl(PPh ₃)] 12r <i>trans</i> -N,P-[Pd{C ₆ H ₄ (Ph)C=N-benzyl}Cl(PPh ₃)] 12s <i>trans</i> -N,P-[Pd{C ₆ H ₄ (Ph)C=N-α-methylbenzyl}Cl(PPh ₃)] 12t <i>trans</i> -N,P-[Pd{C ₆ H ₄ (Ph)C=NH}Cl(PPh ₃)]	MDA-MB-231	72 h: 12a >100 μM 12b >100 μM 12c >100 μM 12d >100 μM 12e 15 μM 12f 40 μM 12g >100 μM 12h 20 μM 12i 16 μM 12j 13 μM 12k 12 μM 12l >100 μM 12m >100 μM 12n >100 μM 12o 1.1 μM 12p >100 μM 12q >100 μM 12r 91 μM 12s >100 μM 12t 1.1 μM Ref. drug: cisplatin 5 μM	Inhibition of cathepsin B. Modification of secondary structure in isolated DNA	Tested vs human umbilical vein endothelial cells (HUVEC)	2014 [72]
	13	[(ClPd(C ₆ H ₄)CH=N(2,6-di- <i>i</i> Pr-C ₆ H ₃)) ₂ (m-Ph ₂ P(CH ₂) ₂ PPh ₂)]	MDA-MB-231	48 h: 0.193 μM	DNA damage. Intrinsic induction of apoptosis via release of cytochrome c, upregulation of PUMA, Bax and downregulation of Bcl-2. Extrinsic induction of apoptosis via activation of caspase 8. Induction of autophagy and G1 cell cycle arrest. Putative anti-cancer stem cell activity	NA	2015 [73]

TABLE 1 (Continued)

Group	Complex no.	Complex designation ^a	Cell line	IC ₅₀	Target/mode of action	Selectivity toward TNBC	Year Refs
Derivatives of pyridine/ pyrazole/imidazole/ pyrrol/triazole and their combinations	14	Pd ₂ Hmtpo	BT-20	Exposure of 2.81 μM for 48 h reduced <50% cell number	DNA fragmentation	NA	2002 [74]
	15	([PdCl(terpy)](sac)·2H ₂ O)	MDA-MB-231 Ehrlich ascites carcinoma (<i>in vivo</i>)	48 h: 46.50 μM <i>In vivo</i> : complex 70% reduction, cisplatin 47% reduction, paclitaxel 59% reduction	Increase of cleaved PARP, caspase 3 activity and pyknotic nuclei	NA	2014 [75]
			MDA-MB-231 MDA-MB-435	72 h: MDA-MB-231: 2.8 μM MDA-MB-435: 11.8 μM	Induction of double-stranded breaks and DNA fragmentation. Induction of apoptosis via activation of caspases 3/7. Modification of secondary structure in isolated DNA	Tested vs primary human aortic smooth muscle cells (HASMC-1 and HASMC-2)	2013 [76] 2017 [77]
	16	[Pd(sac)(terpy)](sac)·4H ₂ O	MDA-MB-231 Ehrlich ascites carcinoma (<i>in vivo</i>)	72 h: 0.09 μM <i>In vivo</i> : complex 68% reduction, cisplatin 33% reduction, paclitaxel 69% reduction	Disruption of tubules. Apoptosis <i>via</i> DR4 and DR5	NA	2011 [78]
			MDA-MB-231		Induction of ROS and DNA damage	NA	2014 [79]
			MDA-MB-231 MDA-MB-435	72 h: MDA-MB-231: 20.57 μM MDA-MB-435: 18.30 μM	Induction of apoptosis	Tested vs primary human aortic smooth muscle cells (HASMC-1 and HASMC-2)	2017 [80]
	17	17a <i>cis</i> -[Pd(κ ² -N,N'-[1-(CH ₂) ₂ NMe ₂]pzol)Cl ₂]	MDA-MB-231	72 h: 17a >100 μM 17b >100 μM 17c 16.2 μM Ref. drug: cisplatin 6.5 μM	Modification of secondary structure in isolated DNA	NA	2011 [81]
		17b <i>cis</i> -[Pd(κ ² -N,N'-[1-(CH ₂) ₂ NMe ₂]-3,5-Me ₂ -pzol)Cl ₂]					
		17c [Pd(κ ² -C,N,N'-[1-(CH ₂) ₂ NMe ₂]-3-(C ₅ H ₄)-5-Ph-pzol)]Cl ₂]					
	18	18a 5-Deoxy-1,2-O-isopropylidene-5-(4-(2-pyridyl)-1H-1,2,3-triazol-1-yl)-α-D-xylofuranose palladium(II) chloride 18b 3-O-Benzyl-5-deoxy-1,2-O-isopropylidene-5-(4-(2-pyridyl)-1H-1,2,3-triazol-1-yl)-α-D-xylofuranose palladium(II) chloride 18c Methyl-5-deoxy-2,3-O-isopropylidene-5-(4-(2-pyridyl)-1H-1,2,3-triazol-1-yl)-β-D-ribofuranose palladium(II) chloride 18d 6-Deoxy-1,2:3,4-di-O-isopropylidene-6-(4-(2-pyridyl)-1H-1,2,3-triazol-1-yl)-α-D-galactopyranose palladium(II) chloride	MDA-MB-231	48 h: 18a 9.9 μM 18b 7.9 μM 18c NA 18d 12.1 μM Ref. drugs: doxorubicin <1 μM cisplatin <1 μM	NA	NA	2012 [82]

TABLE 1 (Continued)

Group	Complex no.	Complex designation ^a	Cell line	IC ₅₀	Target/mode of action	Selectivity toward TNBC	Year Refs
	19	19a ([Pd(bpma)(sac)](sac)·2H ₂ O) 19b ([Pd(bpma)Cl](sac)·2H ₂ O)	MDA-MB-231	72 h: 19a 9.3 μM 19b 4.2 μM	Induction of apoptosis via Fas death receptor. Increase of cleaved PARP and caspase 3 activity	NA	2013 [83]
	20	20a [Pd(bpy)(hmbt)]Cl 20b [Pd(phen)(hmbt)]Cl	MDA-MB-231	120 h: 20a 45.96 μM 20b 4.85 μM Ref. drug: cisplatin 32.0 μM	NA	NA	2014 [84]
	21	21a 3-O-Acetyl-5-deoxy-1,2-O-isopropylidene-5-(4-(2-pyridyl)-1H-1,2,3-triazole-1-yl)-α-D-xylofuranose palladium (II) complex 21b 3-O-Benzoyl-5-deoxy-1,2-O-isopropylidene-5-(4-(2-pyridyl)-1H-1,2,3-triazole-1-yl)-α-D-xylofuranose palladium (II) complex 21c [5-Deoxy-3-O-ferrocenoyl-1,2-O-isopropylidene-5-(4-(2-pyridyl)-1H-1,2,3-triazole-1-yl)-α-D-xylofuranose palladium (II) complex	MDA-MB-231	48 h: 21a >100 μM 21b >100 μM 21c >100 μM Ref. drugs: doxorubicin 0.91 μM cisplatin 0.25 μM	NA	NA	2014 [85]
	22	22a <i>trans</i> -[Pd{[1-{MeO-(CH ₂) ₂ }-3,5-Ph ₂ -(C ₃ HN ₂) ₂ Cl ₂] 22b [Pd{k ² ,C,N}[1-{MeO-(CH ₂) ₂ }-3-(C ₆ H ₄),5-Ph-(C ₃ HN ₂) ₂](μ-OAc)] ₂ 22c [Pd{k ² ,C,N}[1-{MeO-(CH ₂) ₂ }-3-(C ₆ H ₄),5-Ph-(C ₃ HN ₂) ₂](μ-Cl)] ₂ 22d [Pd{k ² ,C,N}[1-{MeO-(CH ₂) ₂ }-3-(C ₆ H ₄),5-Ph-(C ₃ HN ₂) ₂](OAc)(PPh ₃)] 22e [Pd{k ² ,C,N}[1-{MeO-(CH ₂) ₂ }-3-(C ₆ H ₄),5-Ph-(C ₃ HN ₂) ₂](Cl)(PPh ₃)]	MDA-MB-231	72 h: 22a 75 μM 22b 9.5 μM 22c 14.4 μM 22d 9.1 μM 22e 13 μM Ref. drug: cisplatin 6.5 μM	NA	NA	2014 [86]
	23	23a PdCl ₂ (1,2-O-Isopropylidene-α-D-xylofuranose-3,5-(3'-amino)phenyl boronate) 23b PdCl ₂ (1,2-O-Cyclohexylidene-α-D-xylofuranose-3,5-(3'-amino)phenyl boronate) 23c PdCl ₂ (1-O-Benzyl-2,3-O-isopropylidene-α-L-sorbofuranose-4,6-(3'-amino)phenyl boronate) 23d PdCl ₂ (1,2:5,6-Di-O-isopropylidene-D-mannitol-3,4-(3'-amino)phenyl boronate) 23e PdCl ₂ (1,2:5,6-Di-O-cyclohexylidene-D-mannitol-3,4-(3'-amino)-phenyl boronate) 23f structure given in supplementary data S2 23g structure given in supplementary data S2	MDA-MB-231	48 h: 23a 8.58 μM 23b 8.57 μM 23c 34.76 μM 23d 30.37 μM 23e 15.00 μM 23f >100 μM 23 g 47.62 μM Ref. drugs: cisplatin 7.21 μM Doxorubicin 1 μM	Intercalation between strands of isolated DNA	Tested vs normal human embryonic kidney cells (HEK-293T)	2015 [87]

TABLE 1 (Continued)

Group	Complex no.	Complex designation ^a	Cell line	IC ₅₀	Target/mode of action	Selectivity toward TNBC	Year Refs
	24	24a [Pd ₂ (tripyl) ₄](BF ₄) ₄ 24b [Pd ₂ (bntrzl) ₄](BF ₄) ₄ 24c [Pd ₂ (hextrzl) ₄](BF ₄) ₄ 24d [Pd ₂ (pegtrzl) ₄](BF ₄) ₄	MDA-MB-231	24 h: 24a 56.7 μM 24b 18.1 μM 24c 6 μM 24d not determined Ref. drug: cisplatin 41.2 μM	Induction of late apoptosis and disruption of cell membrane	Tested vs normal breast epithelial cells (MCF-10A)	2015 [88]
	25	<i>trans</i> -bis-(<i>cis</i> -7a-ethyl-5-methyl-5-phenylselanylmethyl-tetrahydro-pyrrolo [1,2-c]imidazole-1,3-dionato) palladium(II) chloride	MDA-MB-231	72 h: 81.7 μM	Induction of iNOS protein expression and superoxide anion radical production. Decrease of cells migratory potential	NA	2017 [89]
	26	Includes two complexes 26a,b whose structures are given in supplementary data S3	MDA-MB-231	48 h: 26a 2.8 μM 26b 1.5 μM	Induction of apoptosis. Photodynamic therapy	NA	2017 [90]
	27	27a {[Pd(2,2'-bipy)Cl] ₂ (μ-pz)}(ClO ₄) ₂ 27b {[Pd(dach)Cl] ₂ (μ-pz)}(ClO ₄) ₂ 27c {[Pd(en)Cl] ₂ (μ-pz)}(ClO ₄) ₂ 27d {[Pd(2,2'-bipy)Cl] ₂ (μ-4,4'-bipy)}(ClO ₄) ₂ 27e {[Pd(dach)Cl] ₂ (μ-4,4'-bipy)}(ClO ₄) ₂ 27f {[Pd(en)Cl] ₂ (μ-4,4'-bipy)}(ClO ₄) ₂	MDA-MB-231	48 h: 27a 17 μM 27b 26 μM 27c 25 μM 27d 70 μM 27e >100 μM 27f >100 μM Ref. drug: cisplatin 55 μM	Induction of apoptosis and necrosis. G1/S cell cycle arrest	Tested vs normal human fibroblast (MRC-5)	2017 [91]
Derivatives of thiourea	28	28a [PdCl(PPh ₃) ₂ (N,N-dimethyl-N'-benzoylthioureato-k ² O,S)] 28b <i>cis</i> -[Pd(PPh ₃) ₂ (N,N-diethyl-N'-benzoylthioureato-k ² O,S)]PF ₆ 28c <i>cis</i> -[Pd(PPh ₃) ₂ (N,N-diphenyl-N'-benzoylthioureato-k ² O,S)]PF ₆ 28d <i>cis</i> -[Pd(PPh ₃) ₂ (N,N-dibenzyl-N'-benzoylthioureato-k ² O,S)]PF ₆ 28e <i>cis</i> -[Pd(PPh ₃) ₂ (N,N-diethyl-N'-furoylthioureato-k ² O,S)]PF ₆ 28f <i>cis</i> -[Pd(PPh ₃) ₂ (N,N-diphenyl-N'-furoylthioureato-k ² O,S)]PF ₆ 28g <i>cis</i> -[Pd(PPh ₃) ₂ (N,N-dibenzyl-N'-furoylthioureato-k ² O,S)]PF ₆	MDA-MB-231	48 h: 28a 2.15 μM 28b 1.12 μM 28e <0.8 μM 28c,28d,28f,28 g >200 μM Ref. drug: Cisplatin 2.43 μM	NA	NA	2014 [92]
Derivatives of chloroquine/ clotrimazole	29	29a <i>trans</i> -PdCQ ₂ Cl ₂ 29b <i>trans</i> -Pd(CTZ) ₂ Cl ₂ *2/3CH ₂ Cl ₂	MDA-MB-231	24 h: 29a 49 μM, 29b 159 μM	NA	NA	2005 [93]

TABLE 1 (Continued)

Group	Complex no.	Complex designation ^a	Cell line	IC ₅₀	Target/mode of action	Selectivity toward TNBC	Year Refs
Phosphine derivatives and others	30	30a [Pd(ca ₂ -o-phen)Cl ₂] 30b [Pd(dmab)(dppp)Cl]	MDA-MB-435	Complexes (0.1–5 μM) inhibited 90–95% of cell growth relative to cisplatin (5 μM)	NA	NA	2012 [94]
	31	31a <i>trans</i> -[PdCl ₂ [P(C ₂ H ₄ COOH) ₃] ₂] 31b <i>trans</i> -[Pd ₂ Cl ₄ [P(C ₂ H ₄ COOH) ₃] ₂]	MDA-MB-231	24 h: 31a 8.1 μM 31b 30.5 μM Ref. drug: cisplatin 63 μM	NA	NA	2016 [95]
Derivatives of thiosemicarbazones	32	chloro, mono(phenanthrenequinone thiosemicarbazono) pal- ladium(II) dimethyl formamide solvate	MDA-MB-231 BT-20	Exposure to 3 μg/ml for 72 h reduced cell viability to 15% (MDA-MB-231) and 36% (BT-20)	NA	Tested vs 21 NT (mortal mammary epithelial cells (21NT) and normal breast epithelial cells (MCF-10A)	2005 [96]
	33	2-acetyl pyridine 4N-ethyl thiosemicarbazone palladium(II) complex	MDA (type not further specified)	96 h: ~ 0.7 μM Ref. drug: cisplatin: ~2.5 μM	NA	NA	2007 [97]
	34	3,4-difluoroacetophenone-thiosemicarbazone palladium(II) complex	MDA-MB-231	Exposure to 10 μg/ml for 48 h reduced cell viability to 50%	NA	NA	2013 [98]

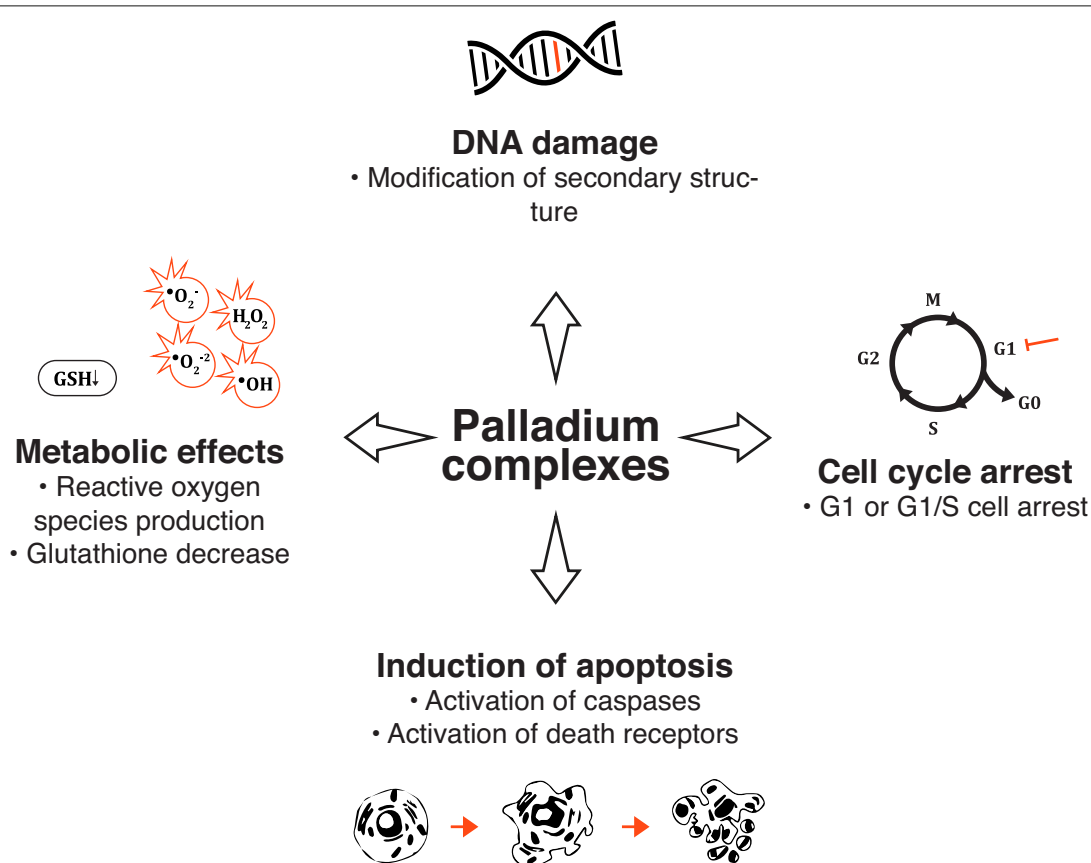
Abbreviations: 2,2'-bipy, 2,2'-bipyridyl; 4,4'-bipy, 4,4'-bipyridyl; BENSpm, *N*¹,*N*¹¹-bis(ethyl)norspermine; bpma, bis(2-pyridylmethyl)amine; bpy, 2,2-bipyridine; ca₂-o-phen, bis(cinnamaldehyde)-o-phenylenediimine; CQ, chloroquine; CTZ, clotrimazole; dach, *trans*-(±)-1,2-diaminocyclohexane; dmab, dimethylbenzylamine; dppe, 1,2-bis(diphenylphosphino)ethane; dppp, (diphenylphosphine)propane; en, ethylenediamine; Et, ethyl; Et₂dasa, diaminosuccinate diethyl ester dihydrochloride; H₂dasa, diaminosuccinic acid; hmbt, 2-(2'-hydroxy-5'-methylphenyl)-benzotriazole; Hmtpo, 5,7-dihydro-7-oxo-5- methyl[1,2,4]triazolopyrimidine; iPr, isopropyl; Me, methyl; MeO, methoxy; NSpd, norspermidine; OAc, acetyl-oxy; pegtrz, (1,3-bis(1-(2-(2-methoxyethoxy)ethyl)-1H-1,2,3-triazol-4-yl)benzene) ligand; Ph, phenyl; Phen, phenanthroline; PPh₃, triphenylphosphine; Put, putrescine; Py, pyridine; pz, pyrazine; sac, saccharinate; Spd, spermidine; Spm, spermine; t-bu, tert-butyl; TCEP, tris(2-carboxyethyl)phosphine; terpy, 2,2':6',2''terpyridine; tripy, 2,6-bis(pyridin-3-ylethynyl)pyridine).

^aComplex designation given by the original authors.

similarities of their main ligands, including ethyl diamine, biogenic polyamines, benzyl amines/imines, pyridine/pyrazole/imidazole/pyrrol/triazole, chloroquine/clotrimazole, phosphine, thiourea and thiosemicarbazones. The IC₅₀ values in a TNBC cell line and cancer selectivity (when present) were also reviewed, along with the target and/or mode(s) of action whenever known.

The wide range of distinct palladium-based compounds reported to date as potential anticancer agents against TNBC, as well as the great variability in the cytotoxicity evaluation studies, hinder the establishment of accurate SAR. Nevertheless, some conclusions can be drawn specifically regarding their biological effects and potency (Table 1): (i) the complexes comprising amine ligands [linear alkylamines including biogenic polyamines and benzyl-amines/imines (**C1–C13**)] were shown to be predominantly DNA-damaging agents through covalent binding of the metal ion to the nucleophilic nitrogen atoms of the DNA bases (mainly the purines) and, for some complexes with modified biogenic polyamines (**6**), binding to glutathione was also evidenced; (ii) for some of the benzyl-amine/imine complexes (**C13**), the induction of apoptosis was reported; (iii) the derivatives of pyridine/pyrazole/imidazole/pyrrol/triazole (**C14–C27**) were shown to be responsible for the induction of apoptosis and necrosis. In turn, for a large number of investigated palladium complexes displaying promising antineoplastic properties [e.g., S-containing complexes of thiourea (**C28a–g**) or

thiosemicarbazones (**C32–C34**)] the mode of action, at the molecular level, is still unknown; (iv) Regarding the choice of ligands for the Pd(II) complexes with activity against TNBC, they are either N-containing polydentate molecules and/or sulfur-moieties [Pd(II) having a higher affinity for sulfur vs nitrogen] to compensate for the high lability of these compounds, thus ensuring intracellular stability and avoiding drug inactivation; (v) Considering the *in vitro* potency toward TNBC cell lines, almost one-third of the studied compounds exhibit IC₅₀ values between 0.1 and 5 μ M, thus having an activity comparable or superior to the reference Pt(II) drug cisplatin. In particular, compounds with ligands such as biogenic polyamines (**C4b,c**; **C5a–c**; **C5e**; **C6**), thiourea (**C28a,b,e**) and thiosemicarbazones (**C33**), as well as some complexes with either ethyl diamine (**C1a,b**), benzyl amine/imine (**C9a–c**; **C10c,d**; **C11c,g,h,k,l**; **C12o,t**; **C13**) and pyridine/pyrazole/imidazole/pyrrol/triazole (**C14**; **C15**; **C16**; **C19b**; **C20b**; **C26a,b**) deserve further attention to verify and study their antitumor properties; (vi) In addition, even though there have already been reported syntheses of Pd(IV) complexes [49] that could be theoretically suitable for oral administration, up to now only Pd(II) complexes have been screened regarding their activity against TNBC cell lines; (vii) The moderate hydrophilicity of Pd(II) complexes points to an intravenous administration for the compounds developed so far. With a view to increasing water solubility, aiming at a most desired oral



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FIGURE 2

Different mechanisms of action described for palladium(II)-based complexes in triple-negative breast carcinoma.

administration, highly advantageous for patient compliance, several combinations of ligands have been tried, going from just one type of molecule within the first coordination sphere of the metal(s) to more than one kind of binding atoms and bridging moieties between them. These structural features are also aimed at tailoring the overall flexibility and stereochemistry of the compound, which are expected to facilitate its transport and enhance its interaction with the target(s). Furthermore, the nature of the leaving ligands – usually chlorides or acetoxy groups [$\text{H}_3\text{C}(\text{C}=\text{O})\text{O}-$] – and their number and position in the molecule (*cis* or *trans*) are relevant features, because they regulate the drug activation mechanism inside the cell, which occurs through chloride hydrolysis, in low $[\text{Cl}^-]$ intracellular medium, followed by aquation, to yield an active drug prone to bind to its target(s) (e.g., DNA); (viii) Interestingly, a variety of Pd(II) complexes investigated so far (**C5e**; **C6**; **C7**; **C12o**; **C15**; **C16**; **C23**; **C24**; **C27**; **C32**) have already exhibited cancer selectivity toward TNBC, coupled to less deleterious effects toward noncancerous cells.

Chemotherapeutic mechanisms of Pd(II) complexes against TNBC

TNBC is a breast cancer subtype that resists conventional chemotherapy and surgical strategies, yielding unsatisfying clinical results and compelling the need for the search for and development of mechanism-based approaches. The profound knowledge of signaling transduction pathways involved in the regulation of cell proliferation and survival are particularly important because most of the alterations occur in effector(s) of these signaling pathways during carcinogenesis. Such effectors are protein kinases, transcription factors and receptors known to maintain homeostasis. Thus, any abnormal activation or silencing of these effectors as well as their downstream signaling could contribute to uncontrolled cell growth leading to cell malignant transformation. This section briefly summarizes the possible mechanisms of palladium complexes and their chemotherapeutic properties in TNBC (Fig. 2).

DNA damage

Palladium complexes can act as a novel class of metal-based agents that bind covalently to the nitrogen bases of DNA, resulting in DNA fragmentation by hindering an adequate DNA synthesis and RNA transcription from the affected DNA areas. DNA damage occurs through formation of crosslinks, preventing DNA strands from being separated for synthesis or transcription, and inducing mispaired nucleotides, leading to mutations [50]. Several palladium complexes, namely **C5e**, **C6**, **C13–C16** and **C19** have been identified to cause DNA conformational changes or fragmentation. The damaged DNA induces the DNA repair mechanisms, which in turn can lead to apoptosis when DNA repair is impeded.

Induction of apoptosis

Several palladium complexes have been associated with proapoptotic events in TNBC, namely **C13**, **C15**, **C16**, **C19**, **C24**, **C26** and **C27**. Apoptosis occurs via activation of two major complex and energy-dependent pathways: the extrinsic (via death receptor) and the intrinsic (via mitochondria) pathways [51]. Some palladium complexes have been linked to the alterations in key

proteins involved in the extrinsic pathway, such as caspase 3 (**C15**, **C19**), caspase 8 (**C13**) and Fas death receptor or tumor necrosis factor (TNF)-related apoptosis-inducing ligand (Trail) receptors 1 (DR4) and 2 (DR5) as reported for **C16**. Influence of palladium complexes on the intrinsic pathway such as cytochrome c release and upregulation of Bax and PUMA (p53 upregulated modulator of apoptosis) genes have also been demonstrated for **C13**.

Cell cycle arrest

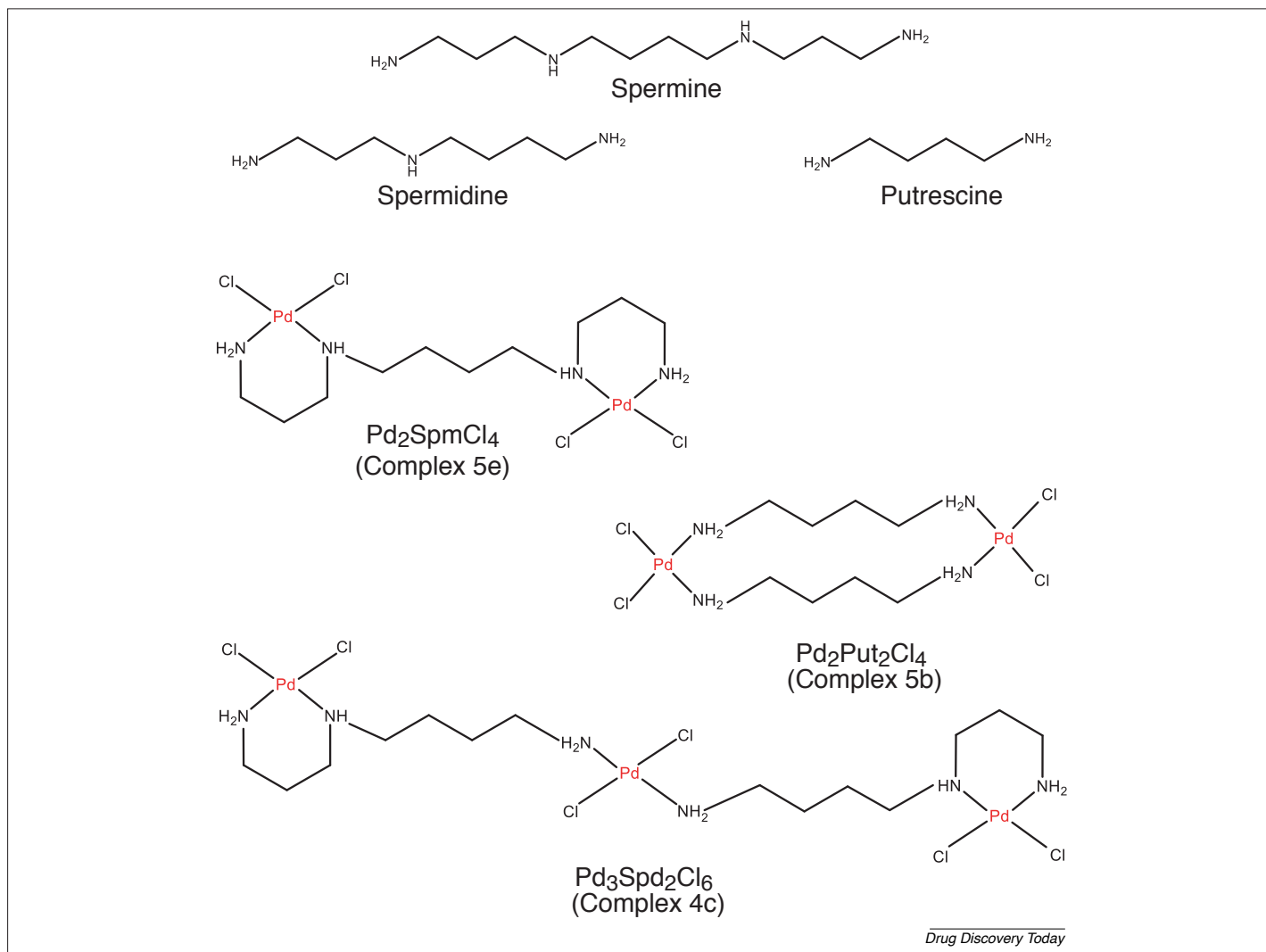
It is well accepted that carcinogenesis is associated with cell cycle deregulation and/or overexpression of growth kinases [52]. Palladium complexes (**C3**, **C13** and **C27**) arrest the cell cycle of TNBC cells in G1 or G1/S phase and these effects can be either direct or indirect. The appearance of the sub-G1 peak that generally represents dead cells has also been reported for **C3** and **C13**. Indeed, DNA damage can lead to cell cycle arrest by triggering the p53 pathway, which, in turn, can lead to initiation of DNA repair or apoptosis.

Increase in reactive oxygen species levels

Reactive oxygen/nitrogen species (ROS/RNS) are formed by metabolic reactions and can interact with biomolecules resulting in oxidation of amino acyl residues in proteins, mutations in DNA and lipid peroxidation, producing more free radicals that increase the risk of mutations [53]. ROS production was reported for **C16** and **C25**. Notwithstanding ROS-induced damage, this can be restored by internal surveillance and repair systems. However, high levels of ROS overwhelm cellular detoxifying systems, stalling cell division and, after prolonged arrest, cells can die from apoptosis. The decrease in glutathione levels, indicating an increase in the intracellular redox status, was reported for **C6**.

Targeting TNBC with palladium–polyamine complexes

The biogenic polyamines putrescine (Put), spermine (Spm) and spermidine (Spd) are low molecular weight organic polycations (Fig. 3) that are biosynthesized intracellularly from arginine and methionine, as well as obtained exogenously through the diet [54]. These biogenic polyamines are essential for eukaryotic cell proliferation and differentiation [55]. Thus, it is not surprising that highly proliferating cancer cells have increased polyamine concentrations, as well as enhanced uptake and biosynthetic mechanisms. Therefore, depletion of the intracellular polyamine pool, through specific polyamine analogs or inhibitors of the polyamine biosynthetic enzymes, is an antineoplastic strategy that has been the object of research for the past 30–40 years. Additionally, the polyamine transport system (for Put, Spd and Spm) is also capable of transporting polyamine-based molecules [56], such as palladium–polyamine complexes (**C4–7**). Therefore, the selectivity of Pd(II)–polyamine complexes toward cancer cells (namely TNBC) could be partially explained by this transport mechanism. Additionally, polynuclear complexes (comprising two or three metal centers) with linear aliphatic amines (Fig. 3) are particularly promising drugs, because they display a very high conformational freedom and variable-length ligands (different bridging chains separating the metal ions within the chelate) [57,58]. This provides stable Pd(II) agents able to interact with the DNA double helix in a very efficient way: via binding to more than one site, leading to the formation of long-range interstrands responsible for a more severe



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FIGURE 3

Structures of the biogenic polyamines and selected palladium–polyamine complexes. Abbreviations: Put, putrescine; Spd, spermidine; Spm, spermine. Structures are numbered according to molecules organized in Table 1.

damage to DNA (as compared with the mononuclear clinically used drugs cisplatin, carboplatin and oxaliplatin), and therefore inducing cell death to a larger extent [59]. However, more-detailed processes might be involved, and this needs further elucidation.

Concluding remarks

TNBC is the breast cancer subtype with the poorest prognosis, owing to its aggressive biology, high metastatic ability and limited pharmacological treatment options with no targeted therapy being available to date. Platinum-based chemotherapeutics such as cisplatin, carboplatin and oxaliplatin are one of the most widely used anticancer pharmacological agents in a variety of cancer treatment regimens, including TNBC. However, their therapeutic efficacy is severely limited by deleterious side-effects and development of resistance during treatment. Therefore, novel metal-based drugs acting through nonconventional mechanisms are necessary to improve therapeutic efficiency coupled to minimal toxicity. Indeed, Pd(II)-based complexes represent an appealing alternative with promising properties against TNBC, owing to their higher

activity and lower off-target toxicity as compared with their platinum counterparts. Furthermore, selectivity toward cancer cells ascribed for some Pd(II) coordination compounds offers an additional advantage comparatively to currently used platinum agents against TNBC. Altogether, the reported anticancer activity of Pd(II)-based complexes put them at the forefront of future studies aiming at the development of new drug candidates against TNBC with superior clinical potential.

Conflicts of interest

The authors report no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.drudis.2019.02.012>.

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