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Review

Optimization of cisplatin for the treatment of hormone dependent tumoral diseases
Part 1: Use of steroidal ligands

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ABSTRACT

Platinum complexes such as cisplatin and carboplatin are metal based drugs, which are widely used in cancer chemotherapy. However, in the current therapy of hormone-dependent breast and prostate cancer they are not established. This might be the result of intrinsic and acquired resistance of the tumors during the therapy. Therefore, several attempts were done to design platinum complexes to address these tumors.

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Steroidal and non-steroidal drugs were modified for the coordination to platinum. The mode of action implies a binding to the estrogen receptor, a selective accumulation in the tumor cells and a specific binding to DNA. In part 1 of this review article we describe the use of steroidal ligands to optimize cisplatin for the treatment of hormone dependent tumoral diseases.

1. Cisplatin and carboplatin in cancer treatment

Cisplatin (cDDP) and its less toxic derivative carboplatin (CBDCA) are two of the mostly used drugs in cancer chemotherapy [1]. In several cancer diseases (e.g. ovarian cancer) cDDP or CBDCA, combined with other anti-cancer drugs, produce complete remissions in part of the patients [1,2]. However, with the exception of the advanced and metastatic testicular germ-cell cancer (both seminoma and non-seminomatous tumors), which are curable in a high percentage of patients, the duration of remission is often reduced as a result of the development of drug resistance [1,2].

In the last years the mode of action of anti-tumor active platinum complexes was thoroughly studied on the example of cDDP (refs. [3–6] for reviews). cDDP attacked guanine-rich sequences under preferred formation of [$(NH_3)_2$Pt{d(GpG)}] and [$(NH_3)_2$Pt{d(GpA)}] cross links bending and distorting the DNA [7,8]. These structural changes affect DNA replication and transcription [9–13] and, therefore, cause inhibition of tumor growth.

The circumvention of intrinsic and acquired resistance of tumors against cDDP and CBDCA was a further topic of importance for the development of Pt-complexes (refs. [1,5b,6,13] for reviews). Among the discussed resistance mechanisms – (1) reduction of intracellular drug concentration, (2) increased detoxification by glutathione and metallothionein, (3) increased repair of platinum–DNA adducts, and (4) increased tolerance to platinum–DNA adducts – the first one, mainly caused by a reduced drug influx, is presumably responsible for the resistance of frequently occurring cancer diseases like breast and prostate cancer.

1.1. Breast cancer therapy with cDDP and CBDCA

In the chemotherapy of breast cancer, cDDP and CBDCA are not routinely used [14], though a definite, yet low activity against this tumor was found in single agent trials [15] and combination chemotherapy trials [16] (for review see ref. [17]). Moreover, the combination of Pt-complexes with estrogens or "partial" antiestrogens (i.e. antiestrogens with estrogenic side effects) can be of interest for breast cancer therapy. Lippard et al. [18] observed that 17β-estradiol (E_2) significantly increased the cytotoxic activity of cDDP and CBDCA in tests on the estrogen receptor positive ER^+ MCF-7 breast cancer cell line.

1.2. Prostate cancer therapy with cDDP and CBDCA

The same situation is observed in prostate cancer (PC) therapy with cDDP, CBDCA and other cytotoxic drugs, in which only moderate effects were achieved on hormone-refractory tumors, with response rates between 27 and 41% [19,20]. Recently, attention was drawn to the benefit of satraplatin (JM-216), an orally administrable Pt-complex, in the treatment of this tumoral disease [21].

Isaacs [22,23], Huben and Murphy [24] as well as Ludwig [25] suggested that a concomitant administration of cytotoxic agents might improve the therapeutic effect of an endocrine therapy of prostate cancer and would lead to a prolonged remission period. A randomized, prospective clinical study on a combination of castration (causing androgen ablation) and cDDP showed that after 6 weeks the number of patients with progressing tumors was much smaller in the group receiving both treatments than in the group with castration alone [26]. Pre-clinical experiments of von Angerer et al. [27] revealed that the activity of the "partial" antiestrogen zindoxifene on the androgen receptor-positive (AR^+) Dunning R3327-G PC of the rat – presumably caused by reduction of the androgen level to castration values due to its residual estrogenic potency – could also be enhanced by simultaneous administration of cDDP (to the mode of action of zindoxifene compare Schneider et al. [28,29]).

2. Strategies of selective drug delivery

The therapeutic facts reported in Section 1 induced scientists to search for Pt-complexes which were more active on breast and prostate cancer than cDDP or CBDCA and were superior to the established endocrine therapeutic measures (see refs. [30–34] for reviews). They strived for Pt-complexes, which delayed or obviated the development of hormone-refractory breast and prostate cancer.

A common approach to develop selectively acting anti-tumor drugs makes use of the "drug targeting concept".

One of the first attempts to develop Pt-complexes acting according to this concept was the synthesis and evaluation of several cis-[dipeptidester]dichloroplatinum(II) compounds by Beck et al. [35]. It was assumed that the complexes were enriched in tumor cells due to an increased uptake of amino acids, peptides and proteins by the latter. The pharmacology of these compounds was thoroughly studied [36–42].

In the following years, further strategies of selective drug delivery were used in the design of new Pt-complexes for treatment of cDDP- and CBDCA-resistant cancer diseases (see ref. [43] for review). To this group of drugs belong: (1) Non-toxic prodrugs, which are enzymatically transformed in tumor tissues into the anti-tumor active drugs. (2) Compounds with macromolecular carrier ligands, which suffer passive tumor targeting based on the EPR effect (enhanced permeability and retention effect observed in tumors). (3) Compounds targeted towards cellular DNA including intercalators and groove binders. (4) Compounds acting via receptor-mediated targeting.

Pt-complexes with ER-affinic carrier ligands belonging to group 4 are the topic of this review. In the last years compounds of this type were synthesized in large numbers and tested on their usefulness in the treatment of breast and prostate cancer endowed with ERs (see part I, Section 5 and part II of this review).

Instead of Pt-complexes like cDDP and CBDCA other cytotoxic entities, e.g. radioisotopes, nitrogen mustard and (2-chloroethyl)nitrosourea, were also introduced into ER-affinic carrier ligands [44a–c]. One of these compounds, estramustine phosphate, is successfully used in the therapy of prostate cancer [44a,45].

3. Selection of estrogen receptor-affinic carrier ligands for platinum complexes with a specific activity against breast and prostate cancer

In the new Pt-complexes, whose design is based on the "drug targeting concept", cDDP, CBDCA and other Pt-complexes were linked to steroidal or non-steroidal estrogens, which served as carrier ligands. It was supposed that such complexes acted more selectively than cDDP and CBDCA against cancers containing ERs (e.g. breast and prostate cancer) because of their capability to accumulate in the

tumor cells due to their binding to the receptor (refs. [43,44a,b,46] for reviews).

3.1. Breast cancer

Pt-complexes with ER-affinic ligands have been predominantly developed for the treatment of breast cancer. Analogous activities on the field of prostate cancer are only at their beginning.

3.1.1. Selective uptake of estrogens by breast cancer cells

In the last years the capability of steroidal and non-steroidal estrogens for an ER-mediated enrichment in breast cancer cells and their utilization as carrier ligands in cytotoxic drugs, e.g. Pt-complexes, were shown in numerous studies (refs. [44a–c] for reviews). According to Otto [47] E_2 – the most used carrier ligand – is selectively absorbed by ER^+ MCF-7 but not by ER^- MDA-MB-231 breast cancer cells (E_2 concentration 0.5 nM, incubation time 1 h, intracellular concentration 20 nM in ER^+ MCF-7 cells, 3 nM in ER^- MDA-MB-231 cells). The used steroidal estrogens and the kind of their linkage to the Pt-pharmacophore are evident from Sections 4 and 5, part I of this review. The non-steroidal Pt-complexes are described in part II of this review.

3.1.2. Breast cancer-inhibiting activity of estrogens

In the pharmacological evaluation of ER-affinic Pt-complexes it must be taken into account that estrogens themselves possess anti-breast cancer activity. As early as 1944 estrogens were successfully introduced into breast cancer therapy by Haddow et al. [48]. Later it was shown that estrogens like E_2 and diethylstilbestrol (DES) inhibited the growth of the ER^+ MXT-M-3,2 breast cancer but not that of the ER^- MXT-M-3,2 (ovex), both implanted into intact female mice [49,50]. In tests on ovariectomized mice bearing an ER^+ MXT-M-3,2 breast cancer graft – mimicking the postmenopausal situation – estrogens caused a stimulation of tumor growth in low dose range ($\leq$0.37 μmol/kg) followed by an inhibition with increasing dosage (e.g. T/C = 22% at 1 μmol/kg) as shown on the example of DES (compare Fig. 2 and Table 3 in ref. [49]). DES markedly inhibited also the proliferation of ER^+ MCF-7 and ER^- MDA-MB-231 breast cancer cells [51], as well as that of ER^+ and an ER^- murine cell lines derived from the MXT-M-3,2 and MXT-M-3,2 (ovex) breast cancer [49], but only at the very high concentration of 10 μM [51]. In contrast to this, E_2 proved to be inactive on the ER^+ MCF-7 breast cancer cell line up to a concentration of 10 μM [52]. These facts suggested that the use of DES instead of E_2 as carrier ligand in Pt-complexes could enhance the anti-breast cancer activity of the latter due to the inherent cytotoxic potency of DES.

3.1.3. Estrogen receptor α and β (ER_α, ER_β)-selective ER_α and ER_β agonists

Recently, besides of the classical estrogen receptor α (ER_α), a further estrogen receptor, ER_β, was reported to be involved in the growth modulation of breast and other target tissues and also of breast cancer [53–55]. The differing distribution of the two estrogen receptors in target cells and the biological effects caused by agonistically or antagonistically acting ER_α and ER_β ligands are thoroughly described by Gustafsson et al. [53–55].

Increased expression of ER_β in ER_α^+ breast cancer correlated with a better clinical outcome after endocrine therapy [56]. This is due to the fact that ER_β can down regulate the expression of a defined group of ER_α/estrogen-stimulated genes involved in cell cycle regulation and DNA replication [56,57]. In accordance with this, Helguero et al. [58] observed in experiments on the ER_α- and ER_β-containing murine mammary epithelial cell line HC11 a stimulation of cell proliferation by the ER_α-selective agonist 4,4′,4″-(4-propyl-(1H)-pyrazole-1,3,5-triyl)trisphenol (PPT) and a growth inhibition as well as an induction of apoptosis by the ER_β-selective agonist 2,2-bis(4-hydroxyphenyl)propionitril (DPN; formulae of some selective ER_α and ER_β agonists see Fig. 1). E_2 did not influence the growth of HC11 cells presumably due to its combined actions on ER_α (proliferation) and ER_β (growth inhibition, apoptosis). The authors suggest that ER_β agonists are a useful additional tool in the treatment of breast cancer, which has up to now only focused on inhibition with ER_α antagonists. Hartman et al. [59] engineered ER_α^+ T47D breast cancer cells to express ER_β and implanted these cells orthotopically into immunodeficient mice for tests with E_2. In contrast to the parental, not ER_β expressing T47D tumors the growth of the resulting ER_α^+/ER_β^+ T47D-tumors was significantly inhibited by E_2. In the latter tumor the expression of the pro-angiogenic factors VEGF (vascular endothelial growth factor) and PDGF-β (platelet-derived growth factor β) was also strongly

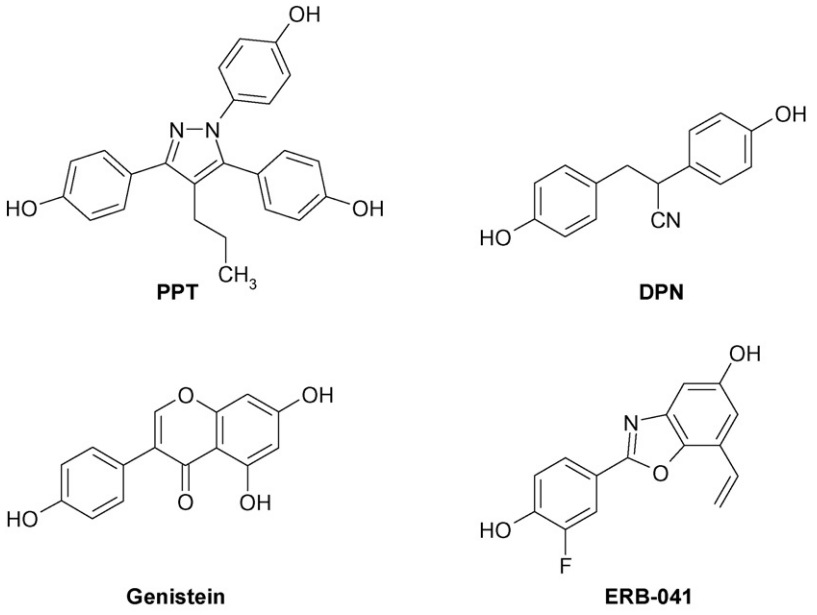


Fig. 1. Selective ER_α and ER_β agonists [58,61,63].

decreased accompanied by a significantly reduced number of intratumoral blood vessels. The ER_β-mediated anti-angiogenic effect of estrogens like E_2 impairs tumor expansion. These authors also conclude that ER_β is an interesting therapeutic target in breast cancer cells and therefore ER_β-selective agonists are potential drugs for the therapy of ER_α^+/ER_β^+ breast cancer due to their anti-proliferative and anti-angiogenic properties.

In contrast to ER_β-selective agonists "true" estrogens like E_2 and DES can stimulate and also inhibit the growth of the ER_α^+/ER_β^+ breast cancer, since they are endowed with ER_α – as well as with ER_β-agonistic potency. Therefore, "true" estrogens should cause a dose-dependent tumor growth stimulation followed by an inhibition or inversely, if the ER_α- and ER_β-agonistic activities differ markedly.

It is conceivable that the biphasic effect – dose dependent tumor growth stimulation followed by inhibition – which was observed in the therapy of the hormone-dependent MXT-M-3,2 breast cancer (implanted into ovariectomized female mice) with DES [49], is at first mediated by ER_α (growth stimulation) and than by ER_β (growth inhibition). In fact, ER_α^+ MXT-M-3,2 breast cancer contains also ER_β, as recently shown by Kunde and Hoffmann [60] by proof of expression of ER_β mRNA in this tumor, which was reducible by ovariectomy or therapy with gestagen antagonists. However, further studies are necessary to fully understand the biphasic effect of "true" estrogens on ER_α^+/ER_β^+ breast cancers.

Interestingly, genistein (formula see Fig. 1), a representative of the phytoestrogens, which are gaining importance in breast cancer therapy [61], proved to be an ER_β-selective partial agonist with a selectivity ratio IC_{50} (ER_α)/IC_{50} (ER_β) of 45 [54,62]. Lately, highly selective ER_β agonists like ERB-041 (selectivity ratio IC_{50} (ER_α)/IC_{50} (ER_β) of 220; formula see Fig. 1), which do not trigger classic estrogenic side effects such as uterine stimulation, and their possible clinical applications were described by Harris [63]. Such compounds could be more suitable carrier ligands in Pt-complexes designed for the therapy of ER_α^+/ER_β^+ breast cancer than the hitherto used "true" estrogens. However, corresponding Pt-drugs have not been synthesized. Further ER_α- and ER_β-selective agonists and concepts to the molecular mechanism of their action are described in refs. [54,64b,c].

3.1.4. "Partial" antiestrogens/SERMs

It cannot be excluded that undesired side effects like increased incidence of endometrial cancer and cardiovascular diseases – as observed in the estrogen replacement therapy of postmenopausal osteoporosis [65] – as well as enhanced tumor growth in postmenopausal patients with breast cancer occur in the therapy with Pt-complexes containing "true" estrogens, e.g. derived from E_2 or DES, as carrier ligands.

"Partial" antiestrogens of the DES, HES and 1,1,2-triarylalk-1-ene type, in which the ER_α-agonistic potency is reduced in favour of an antagonistic one, were used as carrier ligands in Pt-complexes to avoid these adverse effects (see part II of this review). Such antagonists inhibit the proliferation-promoting effect of endogenous estrogens in ER_α^+ tumor cells and are therefore anti-tumor active (compare refs. [64,66]).

It had been shown earlier that simple structures like metastilbestrol (3,3′-DES [67,68]) and its dihydro derivative metahexestrol (3,3′-HES [69,70]), isomers of the "true" non-steroidal estrogens DES and HES, (formulae see Fig. 2) were "partial" antiestrogens and active on breast cancer. Several 1,1,2-triarylalk-1-enes with variable numbers and positions of OH groups in the phenyl rings [71] (e.g. 1,1-bis(4-hydroxyphenyl)-2-phenylbut-1-ene, Fig. 3) also proved to be "partial" antiestrogens with anti-breast cancer activity. Representative ligands derived from these two types of estrogens/antiestrogens, which were used as carriers in Pt-complexes, are listed in part II of this review.

"Partial" antiestrogens like 3,3′-DES, 3,3′-HES and several 1,1,2-triarylalk-1-enes can be considered as SERMs ("selective estrogen receptor modulators") due to their marked inhibitory effects on the ER_α^+ breast cancer and their concomitant weak uterotrophic activity.

A further SERM, the anti-breast cancer active 2-phenylindole derivative zindoxifene (formula: Fig. 4), was used as ER-affinic carrier in several Pt-complexes as early as 1988 (see part II of this review as well as refs. [44a,66]). Another Pt-complex containing tamoxifen (formula: Fig. 4) as carrier ligand was described by Vessieres et al. (see part II of this review and ref. [46]). Tamoxifen itself is used as a SERM in the therapy and the prevention of hormone-sensitive breast cancer (see refs. [64] for review). Nevertheless, mixed ER_α-agonistic/antagonistic acting SERMs are not yet optimal carrier ligands. They increase the incidence of endometrial cancer and can give rise to thromboembolic and other complications [54,64,65,72]. Under postmenopausal conditions, they led to a stimulation of breast cancer growth like observed with "true" estrogens in low dose range [49]. The latter side effect was proved for tamoxifen and other therapeutically used SERMs like toremifene and idoxifene (formulae: Fig. 4) in ovariectomized nude mice bearing human ZR-75-1 breast cancer grafts (a model

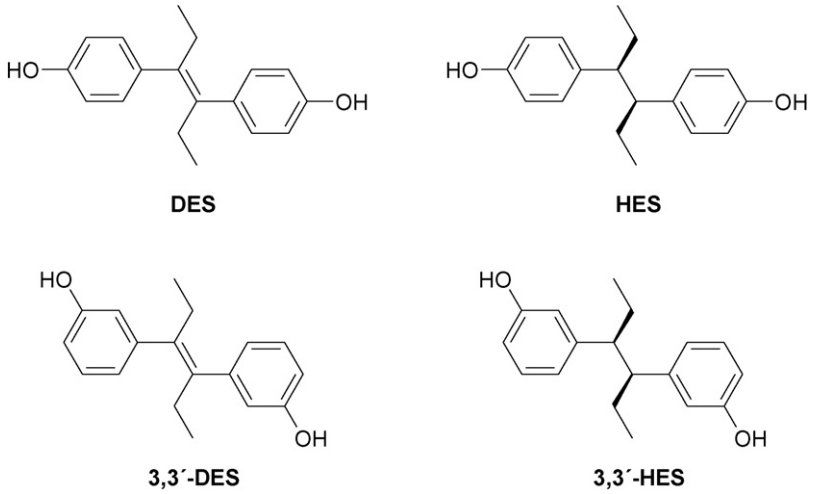


Fig. 2. Non-steroidal estrogens (above) and antiestrogens (below) of the diethylstilbestrol (DES) and hexestrol (HES) type compounds for the therapy of breast and prostate cancer [65,67–70].

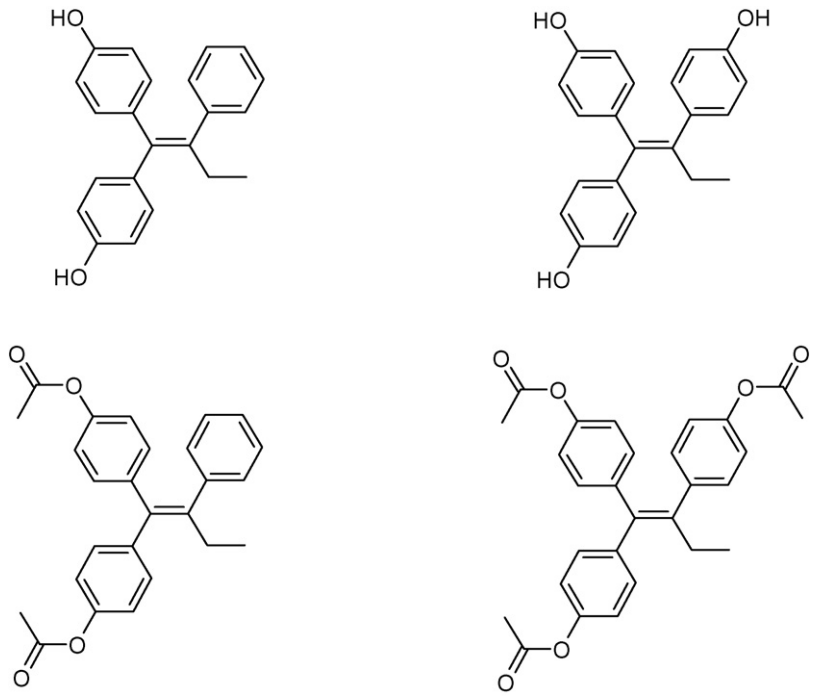


Fig. 3. Ring hydroxylated 1,1,2-triarylbut-1-enes [71] and their O-acetyl derivatives – compounds with anti-breast and anti-prostate cancer activity.

which mirrored the postmenopausal situation) by Gutman et al. [73].

It is absent in the SERM 2,3-diaryl-2H-1-benzopyran (EM-652, formula: Fig. 4), which caused no stimulatory effects on breast and endometrium tissues despite of its very strong ER_α affinity (RBA = 291%, breast cancer cytosol). EM-652 protected bone loss and strongly inhibited the growth of breast and endometrium cancer in animal models [74] and could therefore be a potent carrier ligand for ER-affinic Pt-complexes. In contrast tamoxifen is a poor carrier ligand (RBA = 0.92%, breast cancer cytosol [74]), which was confirmed by a study of Otto [75]. She showed that tamoxifen was not enriched in ER^+ MCF-7 breast cancer cells if used in the anti-tumor active concentration of 1 μM.

3.1.5. *Use of androgens and gestagens as carrier ligands in Pt-complexes*

Besides of estrogens and SERMs various steroid hormones like androgens and gestagens, which themselves possess activities against breast cancer, were constituents of new Pt-complexes (for the steroids used see Section 5, part I of this review; indications

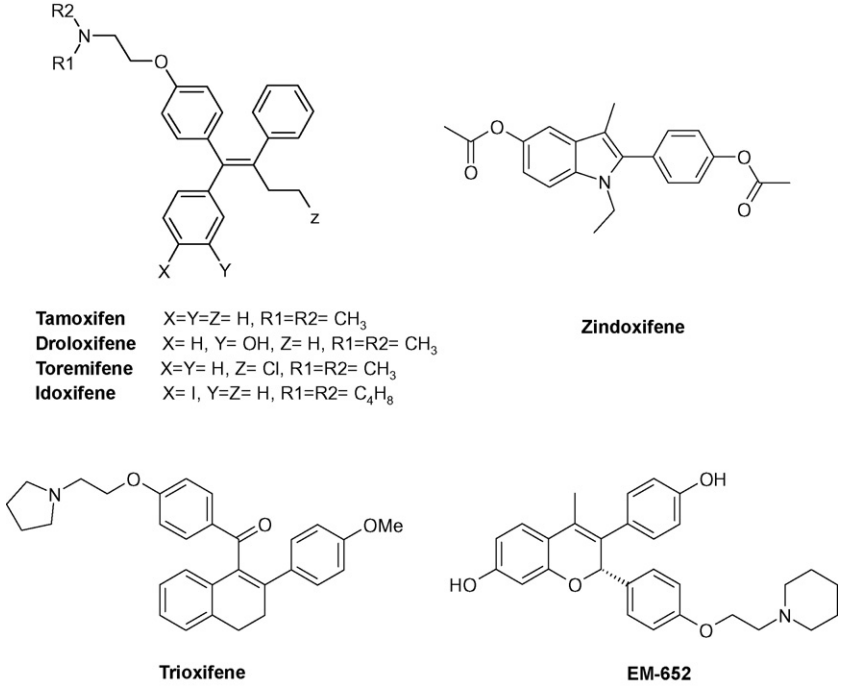


Fig. 4. Anti-breast or anti-prostate cancer active SERMs [44a,66,71,74].

concerning the steroid hormone receptor pattern in breast cancer see refs. [53,76–80] and the endocrine therapy with hormones and antihormones see refs. [30–33,81]).

3.2. Prostate cancer

ER_β-affinic and -agonistically acting Pt-complexes are of particular interest as drugs for the therapy of prostate cancer due to the presence of ER_β in the androgen-dependent (i.e. AR^+) as well as -independent (i.e. AR^-) prostate cancer [53,82–85] and to the growth-inhibiting effect of estrogens on both tumor types under in vivo conditions [82,85,86].

3.2.1. Selective uptake of estrogens by ER_β-containing prostate cancer cells

Studies on the receptor-mediated enrichment of estrogens in prostate cancer cells as performed with breast cancer cells are missing.

3.2.2. Prostate cancer inhibiting activity of estrogens

From recent works of Corey et al. [82,85] it is obvious that estrogens like E_2 inhibit the prostate cancer not only by suppression of the hypothalamus–hypophysis axis resulting in a reduction of the testosterone level, but also by mechanisms not related to androgen ablation. The authors drew this conclusion from experiments in which they observed a significant growth inhibition of human prostate carcinomas, if the tumor fragments were implanted in intact but not in ovariectomized female Balb/c-nu/nu mice. In the latter experiment, E_2 administration led to an inhibition of tumor establishment and to diminished tumor growth similar to what was observed in untreated, intact female mice. The presence of ER_β messages in the prostate cancer xenografts used in this study led the authors to the assumption that the inhibition of prostate cancer growth by E_2 could probably be attributed to its interaction with ER_β. It is described that 5α-androstane-3β,17β-diol (3β-adiol), which is discussed as natural ligand for ER_β, suppresses epithelial proliferation and promotes differentiation of the ventral prostate in rodents [54,87,88]. Therefore, it is assumed that 3β-adiol can be used for the prevention of prostate cancer. The same effects as those of 3β-adiol, inhibition of proliferation and promotion of differentiation, seem to be responsible for the anti-prostate cancer activity of estrogens, since they are also ER_β agonists.

It is noteworthy that in contrast to E_2 the non-steroidal estrogen DES, which was introduced into prostate cancer therapy by Huggins and Hodges as early as 1941 [89], causes moderate, but significant cytotoxic effects on prostate cancer cells [90]. Therefore, DES is more suitable as carrier ligand in anti-prostate cancer active Pt-complexes than E_2.

DES itself can be regarded as a structurally simple drug, which acts on prostate cancer according to the "drug targeting concept" in the same way as ER_β-affinic and -agonistically acting Pt-complexes presumably do. Diethylstilbestrol diphosphate (fosfestrol), a better-tolerated water soluble prodrug of DES, which can be administered intravenously, is even further enriched in prostate cancer via activation by acidic phosphatase [91]. DES is used in the therapy of advanced prostate cancer after failure of surgical or medical castration to control the manifestation of the disease [92–96]. The harmful effect of DES on AR^- PC cells was also demonstrated in a long-term experiment on the Dunning R3327-H PC of the rat, in which the duration of the inhibitory effect until disease progression was significantly extended in comparison to orchiectomy [90]. The marked inhibitory effect of DES on the AR^- PC was also seen in rats bearing implants of the Dunning R3327-H PC relapsed after castration [90]. This tumor model was resistant against androgen ablation indicating an androgen receptor negative status.

The studies suggest that in addition to the moderate cytotoxic potency, evident from cell culture experiments with DES [90], ER_β-mediated processes like modulation of immune response [97], triggering of anti-angiogenesis [98], reduced proliferation and increased differentiation of tumor cells [54] are also responsible for the growth inhibition of AR^- PC after DES administration. The DES effect cannot arise from a simple direct interaction of the drug with the tumor cells (e.g. by interfering with processes of the DNA replication), since marked proliferation-inhibiting effects of DES on the human LNCaP/FGC PC cell line were only determined at a concentration about three times higher than that causing maximal tumor inhibition in animal experiments [90].

3.2.3. "Partial" antiestrogens/SERMs/ER_β agonists

The observation that the therapy with DES is associated with marked side effects like excessive risk for cardiovascular problems [99–102] gave rise to testing better-tolerated DES analogues of the types "partial" antiestrogen and "impeded" estrogen, respectively, e.g. 3,3′-DES and 1,1-bis(4-hydroxyphenyl)-2-phenylbut-1-ene on anti-prostate cancer activity [103] (formulae see Figs. 2 and 3). Interestingly, the two compounds were very active on the Dunning R3327 PC of the rat, despite of their markedly weaker ER-agonistic potency [103]. However, further studies, especially on AR^- PC models, are necessary to prove the equivalence of these compounds to DES.

SERMs can also be potent drugs for the treatment of prostate cancer as first shown on the example of zindoxifene [28,29,44a]. It is remarkable that zindoxifene was even able to delay the relapse of the Dunning R 3327-H PC by 7 weeks in comparison to castration [29]. Tamoxifen produced only marginal inhibitory effects on this tumor [29]. Therefore, zindoxifene was selected as carrier ligand of ER-affinic Pt-complexes for the indication prostate cancer (see part II of this review). Another SERM, toremifene, can prevent the development of prostate cancer in the TRAMP mouse model [104] (i.e. transgenic adenocarcinoma of mouse prostate described in ref. [105]). Gustafsson [53] supposed the chemopreventive efficacy of toremifene to be based on ER_β-agonistic effects inhibiting the proliferation of the prostate epithelium. A further SERM – trioxifene, an ER_α/ER_β-mixed partial agonist – showed anti-metastatic efficacy in tests on PAIII (an $ER_\alpha{}^+/ER_\beta{}^+$ PC)-bearing rats [106].

In the last years, scientists have been more and more interested in the protective role of phytoestrogens against prostate cancer. One representative of these compounds, genistein (formula see Fig. 1), contained in the diet reduced the incidence of poorly differentiated prostatic adenocarcinoma in transgenic mice [105,107] despite of its weak estrogenic potency. Such compounds shift prostatic cancers to a higher differentiation grade, thus bringing the tumor to a less malignant behaviour and improve overall prognosis (for review, see ref. [88] and refs. [82–86] cited therein). Genistein acts also prostate cancer inhibiting by ER_β-mediated down-regulation of the androgen receptor as shown by Bektic et al. [108].

A clue to an important mechanism of the anti-prostate cancer activity of estrogens was given by a study of Joseph and Isaacs [98] with genistein. It significantly inhibited the growth of the androgen-independent Dunning R3327 MAT-Lu PC of the rat via a perturbation of the function of the tumor-associated macrophages (TAM) in the angiogenesis. They observed a diminished TAM number as well as a reduced blood vessel density in the tumors.

Moreover, genistein up-regulated expression of genes with anti-angiogenetic or anti-metastatic properties [109,110].

New results to the function of ER_β direct attention to a possible anti-proliferative role, or to one which promotes apoptosis, and thereby restrains abnormal growth [53,86].

Due to their presumably weak side effects, ER_β agonists like genistein could be interesting carrier ligands for future Pt-complexes designed for prostate cancer therapy.

The studies on the growth-inhibiting activity of "true" and "partial" estrogens, SERMs and ER_β agonists against hormone-sensitive ($AR^+/ER_\beta{}^+$) and -resistant ($AR^-/ER_\beta{}^+$) prostate cancers and their complex mode of action suggest that the use of these compounds as carrier ligands in Pt-complexes will lead to potent drugs.

The strategy of chemical combination of carrier ligand and Pt-pharmacophore without marked reduction or loss of ER affinity and ER-agonistic or ER-antagonistic activity is described in Section 4, part I of this review.

4. Strategy for the design of Pt-complexes containing a steroidal estrogen as pharmacophore

In the new Pt-complexes the estrogenic pharmacophore is responsible for two receptor-mediated processes: (a) Drug accumulation in tumor cells entailing an enhanced impairment of DNA function via formation of intrastrand cross links by the second pharmacophore (Pt-residue). (b) Interference of the estrogenic pharmacophore with growth-modulating processes in the tumors. Therefore, it is essential that the biological properties of both, the estrogenic as well as the cytotoxic component, are retained after their chemical linking.

The obvious choice, the binding of the Pt-pharmacophore by means of an appropriate spacer to one of the two OH groups in steroidal estrogens is not promising, since these groups are involved in the interrelation with the ER. Yet, such an attempt can be made, as ER affinity has been observed in several estrogen or antiestrogen derivatives containing an O-bound cytotoxic residue [44a]. One example is the bis-dichloroacetate of the non-steroidal, "partial" antiestrogen 2,3-bis(2-fluoro-4-hydroxyphenyl)-2,3-dimethylbutane, which showed a significantly better anti-tumor effect than its parent compound on the ER^+ MXT-M-3,2 breast cancer of the mouse [111]. Recently it was reported that dichloroacetate, which induces apoptosis, decreases proliferation and inhibits tumor growth without apparent toxicity, is a promising selective anti-cancer drug [112].

Studies of Katzenellenbogen et al. [113], in which the comparison of the binding affinities of numerous derivatives of E_2 to the ER with that of the parent compound E_2 revealed a relatively high tolerance of the ER for structural modifications of E_2, pointed to suitable positions in the E_2 ring skeleton for the substitution by Pt-pharmacophores. They observed that larger organic residues weakened but not prevented the ligand receptor interaction, if they were linked to E_2 in 7α-, 11β- or 17α-positions and possessed hydrophobic or moderately polar character. Obviously, a sufficiently long distance between the polar group and the core of the steroid is important for a marked drug–receptor binding. Small hydrophobic substituents at positions 4, 12β, 14 or 16α even enhanced the binding affinity of E_2. Substituents in neighbourhood to the phenolic OH group of E_2, which is important for the interaction with the ER, can substantially reduce the binding affinity of E_2 to its receptor by hindering the formation of intermolecular hydrogen bonds between drug and receptor.

Investigations of Jaouen et al. [114] in the field of nuclear medicine concerning the synthesis of agents for diagnostic or therapeutic purposes showed a corresponding relationship between structure and ER affinity in the class of organometallic E_2 derivatives. They observed that the linking of bulky organometallic fragments with rings A or D of E_2 led to compounds with significant ER affinity (although reduced in comparison to E_2), if (a) the OH function was maintained and (b) the substitution took place on the α-face, especially in the 17α-position of the steroid. A short spacer like an ethynyl link between C-17 and the organometallic moiety was useful for an optimal binding to the ER. They concluded that directing the substituent away from the β-face of E_2 reduced steric interference with the 17β-hydroxyl binding pocket of the receptor.

It is conceivable that the linking of the Pt-pharmacophore via a spacer in 7α- or 11β-position of E_2 is accompanied by a change from an ER-agonistic to an ER-antagonistic potency, since E_2 derivatives with a polar side chain in these positions are described to be "true" antiestrogens possessing anti-breast cancer activity [44d]. The synthesis of such Pt-complexes and their evaluation on anti-breast cancer activity could be of interest.

The facts described by Katzenellenbogen et al. [113a,b] and Jaouen et al. [114] have only been partially utilized in the design of steroidal Pt-complexes. From the proposed binding sites for Pt-pharmacophores in the E_2 ring skeleton only positions 4, 16α and 17α have been chosen in the synthesis of steroidal Pt-complexes.

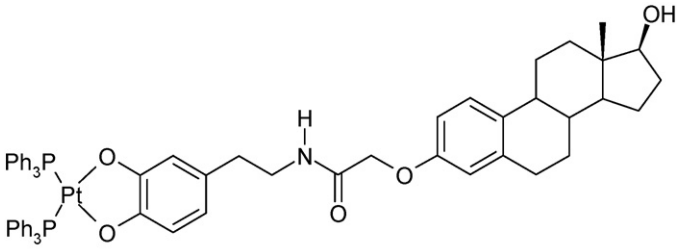
An interesting suggestion of Wang and Lippard [6] is the transformation of Pt(II)-complexes like cDDP or CBDCA into their trans-dihydroxyplatinum(IV) analogues, followed by functionalization with a pharmacophore, e.g. a steroidal antiestrogen, which can contribute to the anti-cancer activity of the former. The pharmacophore is liberated in the tumor cell by reduction of the Pt(IV)-complex to the Pt(II) species. An ER-mediated enrichment of this type of Pt-complex in breast and prostate cancer cells may be achieved by use of appropriately linked estrogens or antiestrogens as pharmacophores.

5. Survey of the synthesized and pharmacologically evaluated steroidal Pt-complexes

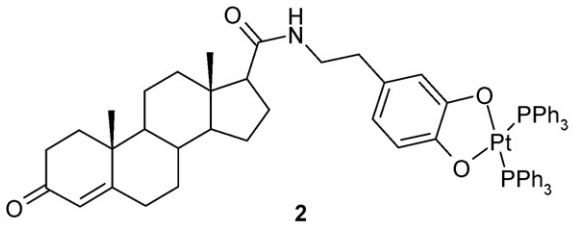
Several groups worked on the field of Pt-complexes containing ligands derived from steroids which were meant to act as carriers transporting the drug selectively into steroid receptor positive cancer cells, particularly of breast and prostate, as described in Sections 2 and 3, part I of this review. In the following, we focus on Pt-complexes with ligands possessing affinity to the ER, for which the greatest amount of data is available, and also to the progesterone and androgen receptor. These receptors are present in significant concentrations in breast and prostate cancer cells and play an important role in the biochemistry of the latter.

5.1. Replacement of the OH groups in positions 3 and/or 17β in the steroid skeleton of E_2 by Pt-pharmacophores

Gandolfi et al. [115a,b] synthesized steroid-catecholatoplatinum(II) complexes to get metallohaptens for metallo immuno assays. Two of the compounds, in which the cytotoxic [4-(2-aminoethyl)-1,2-benzenediolato(2-)-O,O′]bis(triphenyphosphine) platinum(II) residue is attached to the 3-OH group of E_2 via a NH-CO-CH_2-O link (**1**) and to the 17β-carboxylate group of the gestagen 4-androsten-3-one-17β-carboxylic acid (derived from progesterone) by formation of a CO-NH bond (**2**), were tested on the ER^+ MCF-7 breast cancer cell line containing estrogen as well as progesterone receptors. cDDP and the Pt-pharmacophores were used for comparison.



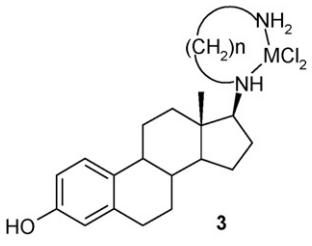
1



2

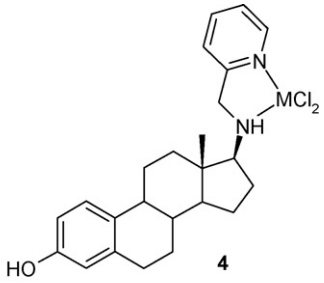
Neither **1** nor **2** surpassed the comparison compounds in their anti-tumor activity. However, it is difficult to interpret these results, since receptor-binding studies are missing. An intracellular release of an active cDDP species and/or the conversion of the coordinated o-catechol into the corresponding active o-quinonoid form were discussed as mode of anti-tumor action (see also ref. [115c]).

Fernández et al. [116] reported on the preparation of cis-$PtCl_2$- and $PdCl_2$-complexes with ethylenediamine, propylenediamine or aminomethylpyridine ligands substituted in 17β-position of 3-hydroxyestra-1,3,5(10)-trien (**3** and **4**) as potential drugs for the therapy of breast, prostate and uterine cancer.



3

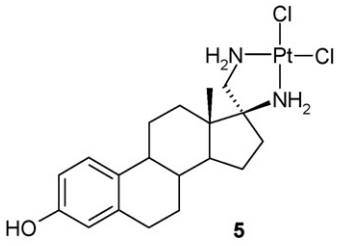
M = Pt(II) or Pd(II); n = 2,3



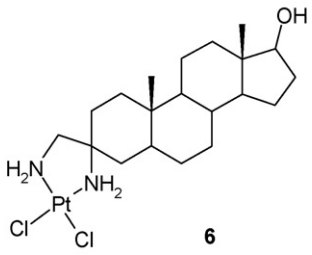
4

M = Pt(II) or Pd(II)

Georgiadis et al. [117] synthesized cis-$PtCl_2$-complexes by reaction of $PtCl_4^{2-}$ with steroids derived in 17- or 3-position with an ethylenediamine moiety, e.g. **5** and **6**. cis-[3-Hydroxyestra-1,3,5(10)-trienyl-17β-amino-17α-methylamino]dichloroplatinum(II) (compound **5**, derived from E_2) possessed a relative binding affinity to the ER of 6.03% (E_2; RBA = 100%). The RBA of compound **6** to the androgen receptor was 1.4%. Tests on breast and prostate cancer were not performed.

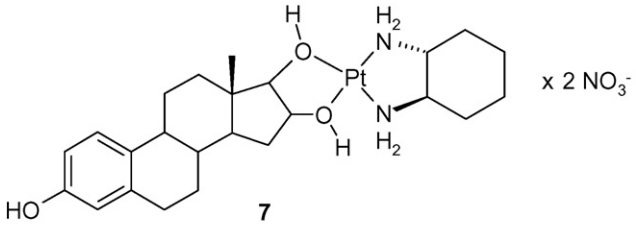


5

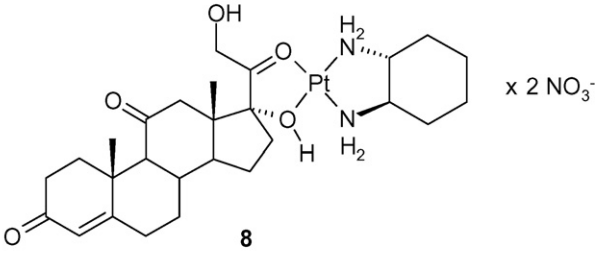


6

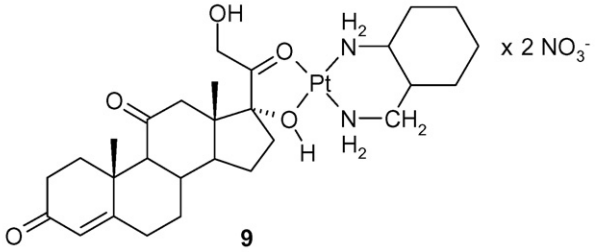
Kidani et al. [118a–c] reported on the synthesis of cationic 1,2-cyclohexanediamine (dach) and 2-(aminomethyl) cyclohexylamine (amcha) Pt-complexes with weakly bound steroids, e.g. estriol (**7**) or cortisone (**8** and **9**) functioning as "leaving groups". These compounds showed marked anti-tumor activity in the in vivo tests on the leukemia L 1210. The evaluation of inhibitory effects on breast and prostate cancer as well as of the binding affinities of these compounds to steroid receptors was not performed.



7

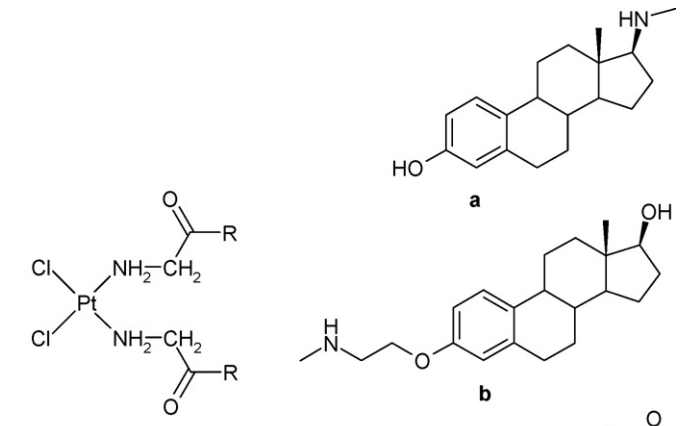


8



9

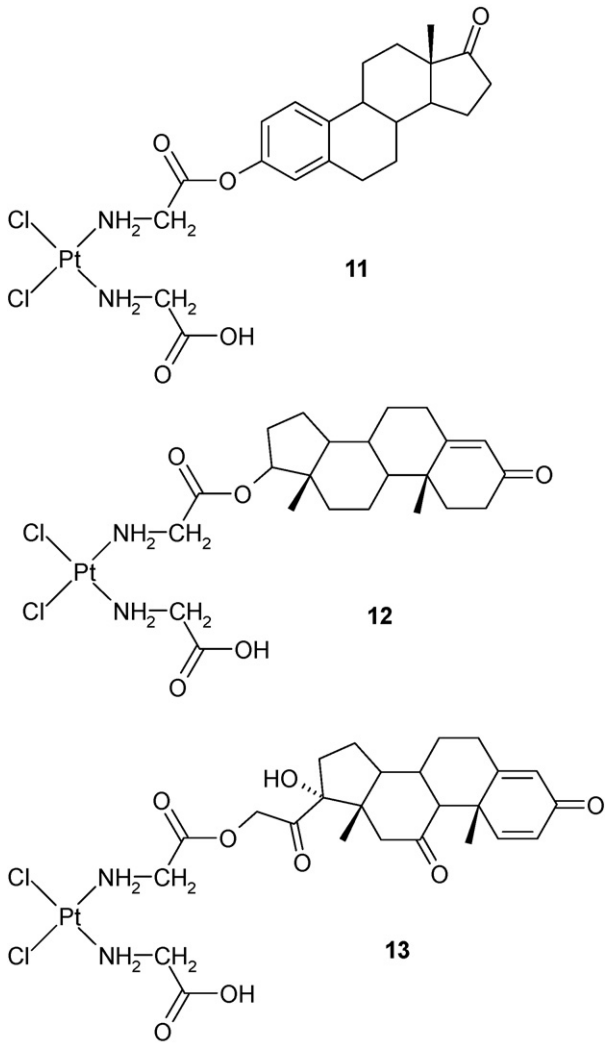
Altman introduced her knowledge on amino functionalized steroids to Beck's group; they transformed such steroids into amides with activated, $PtCl_2$-protected amino acids and peptides to give cis-$PtCl_2$-complexes like **10a–c** [119,120]. The comparative testing of these complexes on ER^+ MCF-7 and ER^- MDA-MB-231 breast cancer cells showed a somewhat stronger sensitivity of the former ER-containing cell line than that of the latter possessing no significant ER levels. In the ER^+ MCF-7 experiment, the antiproliferative effect of **10b** and **10c** at 1 μM was comparable with that of cDDP [119]. However, it is unknown if **10b** and **10c** are endowed with the desired ER affinity.



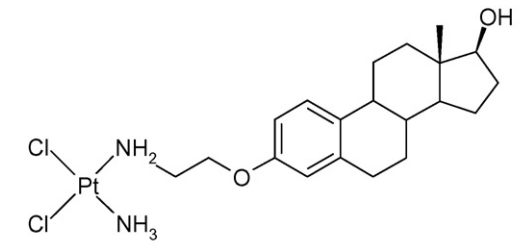
10a-c

R = steroidal carriers a - c

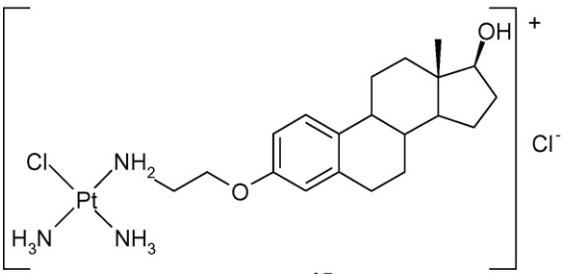
Similarly, estrone, testosterone and prednisone were converted with activated cis-$Cl_2Pt(GlyOH)_2$ into the corresponding esters **11**–**13** (Altman et al. [120]). Pharmacological data of these compounds are not available.



The neutral Pt-complex cis-[$PtCl_2(NH_3)$(3-(2-aminoethoxyestradiol)] (**14**) and the cationic Pt-complex cis-[$Pt(Cl)(NH_3)_2$(3-(2-aminoethoxyestradiol)] (**15**) were also synthesized by Altman et al. and tested on cytostatic activity on the ER^+ MCF-7 breast cancer cell line [120].



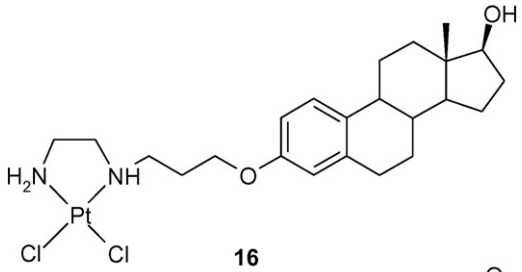
14



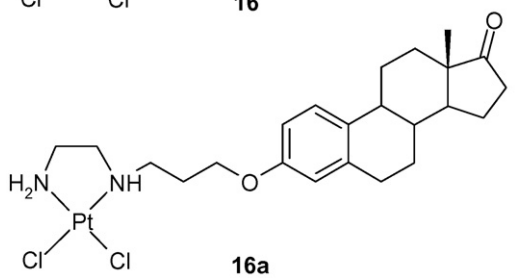
15

No effect was observed for the neutral Pt-complex **14**, whereas the cationic compound **15** was cytostatic at 5 μM with a T/C_{corr} value of ≈40%. The extent of inhibition was comparable with the effect of tamoxifen. Both compounds, **14** and **15**, showed significant inhibitory effects on the L 1210 leukemia cell line. Complex **15** was also active in vivo on the P 388 leukemia of the mouse. The proof of an ER-mediated mode of action of the anti-breast cancer active compound **15** has not been performed.

Cis-[estra-1,3,5(10)trien-17β-ol-3-[oxopropyl-3-(aminoethylamino)]]dichloroplatinum(II) **16** and its estrone analogue **16a** were described by Kourounakis et al. [121]. As expected for complexes whose Pt-pharmacophore is linked with the 3-OH group, the compounds showed only marginal RBAs (**16**: 0.57% and **16a**: 0.98%). Preliminary experiments with **16a** (5 μM) indicated a 71% growth inhibition of the ER^+ MCF-7 breast cancer cell line (cDDP at 5 μM: 76% inhibition).

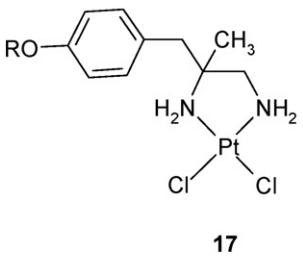


16

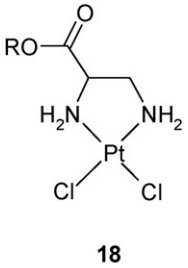


16a

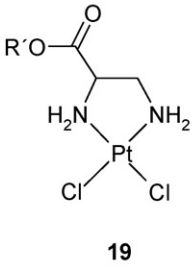
Brunner and Sperl [122] described cis-$PtCl_2$-complexes of 3-hydroxy-17β-[p-(1,2-diamino-2-methyl-prop-3-yl)phenoxy] estra-1,3,5(10)-trien (**17**), 3-hydroxyestra-1,3,5(10)-trien-17β-yl-2, 3-diaminopropionate (**18**), estra-1,3,5(10)-trien-17-one-3-yl-2,3-diaminopropionate (**19**) and also of functional derivatives of the latter compounds.



17

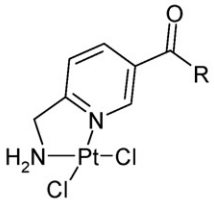


18



19

R: E_2 linked in 17ß-position; R': E_1 linked in 3-position

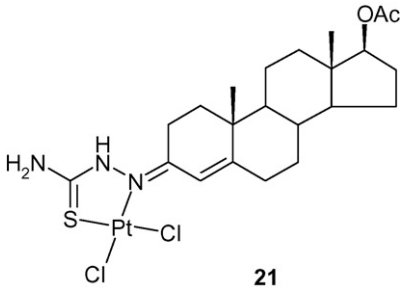


20a: R = E_2 connected in 3-O-position;
20b: R = E_2 connected in 17-O-position.

They evaluated the ER affinity of these complexes and performed comparative tests on the ER^+ MCF-7 and the ER^- MDA-MB-231 breast cancer cell line. Blocking of the 17β-OH group in E_2 caused a marked reduction of the RBA from 100% to 0.08% for **17** and to 0.59% for **18**. Interestingly, the substitution of the 3-OH or 17β-OH group in E_2 by H also decreased the RBA to low values of 3.0% and 3.4%, respectively (cited in ref. [122]). The same phenomenon, a strong reduction of the RBA after change of residues in 3- or 17-position in E_2, was observed by Georgiades et al. [117] for **5** (17-position), Leclercq et al. [126] for **23d** (17-position) and Kourounakis et al. [121] for **16** (3-position). Brunner and Sperl [122] could not observe significantly stronger inhibitory effects of the compounds **17** (T/C_{corr} = 55.4% (51.6%) at 10 μM) and **18** (T/C_{corr} = 68.1% (76.2%) at 10 μM) on the ER^+ MCF-7 breast cancer cell line compared to the ER^- analogue MDA-MB-231 (T/C_{corr} values in parentheses), other than expected for Pt-complexes possessing an ER-affinic carrier. In the case of the more ER-affinic complex **19** (RBA = 5.18%) the ER^- tumor cells were even more sensitive than the ER^+ cells (T/C_{corr} = 52.5% (20.3%) at 10 μM)).

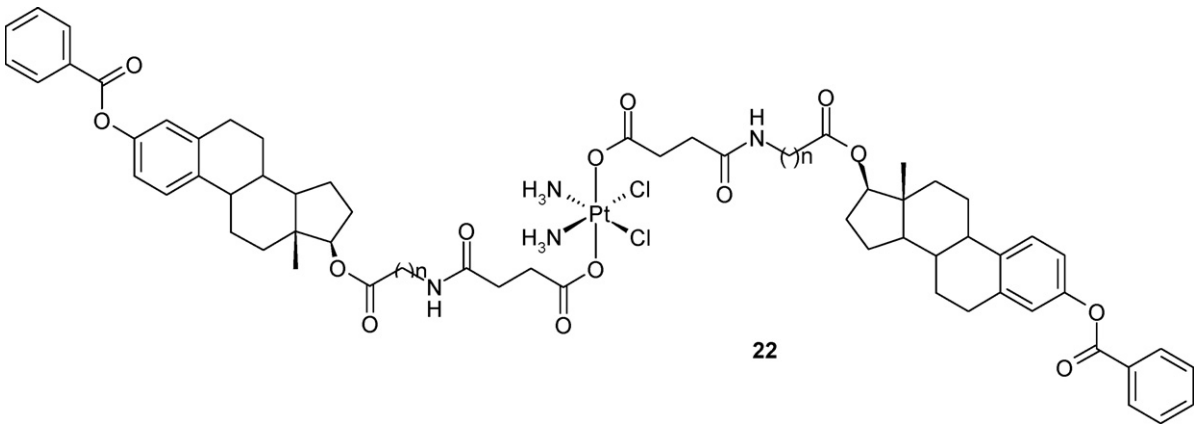
Recently, Schobert et al. [123] reported on active conjugates of [6-amino methylnicotinate] dichloroplatinum(II) with steroids, e.g. E_2 linked in 3-O- (**20a**) and 17-O-position (**20b**), respectively. The most interesting complex **20a** distinctly inhibited the growth of the ER^+ MCF-7 breast cancer cell line, but had little if any effect towards ER^- breast cancer cell lines. The same behaviour against ER^+ and ER^- breast cancer cell lines showed the less effective 17-O-linked isomer **20b**. These results supported the concept of a receptor-mediated accumulation of **20a** and **20b** in ER^+ breast cancer cells, since the latter were significantly bound to the ER_α although to about one order of magnitude weaker than E_2. The authors also studied the ER_α-agonistic potency, which modulate the anti-breast cancer activity in vivo, on the example of the more cytotoxic compound **20a** using the luciferase gene reporter assay. Compound **20a** acted as intact compound and was equipotent to E_2. Therefore, **20a** should be tested in animal experiments on anti-breast and also on anti-prostate cancer activity. It is of interest to note that **20a** strongly interacted with the sex hormone binding globulin (SHBG), which is known to be a specific carrier protein, and only **20b** but not **20a** interacted with DNA (described as mode of action of cDDP) as proved by gel electrophoresis.

A cis-$PtCl_2$-complex of testosterone acetate thiosemicarbazone (see formula **21**) was synthesized and tested in vitro against human ER^+ MCF-7 breast cancer cells by Padhye et al. [124] and showed cytotoxicity comparable to that of cDDP. The compound could be of interest for the therapy of breast and prostate cancer, though studies on the AR-affinity and on prostate cancer cell lines are not available.



21

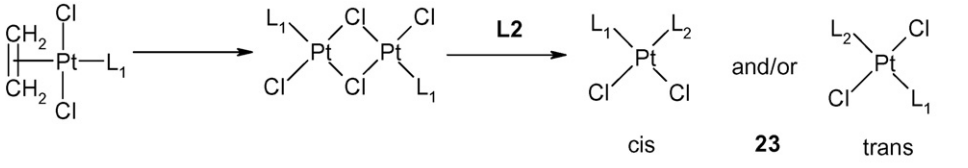
Several estrogen-tethered Pt(IV)-complexes (**22**) were prepared by Lippard et al. [125a–c], inspired by the observation that the inhibition of ER^+ breast cancer cells by cDDP was about 2-fold in the presence of E_2 [18]. This effect was attributed to a receptor-mediated over expression of high-mobility group domain proteins such as HMGB1 by E_2 sensitizing breast cancer cells to cDDP by shielding its major DNA adducts from nucleotide excision repair. The Pt(IV)-complex is reduced in the tumor cell allowing the release of both cDDP and linker-modified estrogen. The validity of this concept was proved for **22** (n = 3) by comparative testing on the ER^+ MCF-7 and ER^- HCC-1937 breast cancer cell lines (IC_{50} = 2.1 ± 0.4 μM versus 3.7 ± 0.9 μM, respectively). The compound was also able to induce over expression of HMGB1. This example provides a novel strategy: namely, using mechanistic insights to aid in the rational design of new complexes in the development of Pt-containing anti-cancer agents.

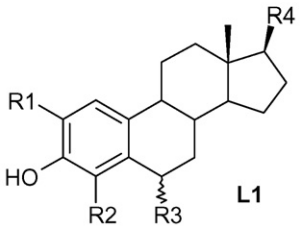


22

5.2. Linkage of the Pt-pharmacophore to the steroid skeleton of E_2 under maintenance of the OH functions in the 3- and 17β-positions

Leclercq et al. [126] studied cis-dichloroplatinum(II) complexes with modified E_2 bearing an NH_2 group in positions 2,4,6 (mixture of 6α and 6β) or 17β (instead of OH; compare Section 5.1, compounds **3–5** and **10a**) as first (L1) and 4-methylpyridine (py^4) or 2,4,6-trimethylpyridine ($py^{2,4,6}$) as second neutral ligand (L2). In the applied synthesis procedure the $py^{2,4,6}$ derivatives were obtained as pure trans-$PtCl_2$–platinum(II) complexes and the py^4 derivatives as non-separable cis/trans-mixtures. Only the latter series is of interest, since the cis-$PtCl_2$–platinum(II) structure is the prerequisite for the formation of intrastrand cross links and with this for cytotoxic properties. All agents were tested on ER affinity by use of rabbit uterine cytosol as receptor source. The RBAs differed in the py^4 series between 0.02% and 4.0%. The most active agents, **23a** (RBA = 4.0%), **23b** (RBA = 2.5%) and **23d** (RBA = 0.75%) of the py^4 series, were also evaluated in the whole cell assay (ER^+ MCF-7) for ER affinity. Comparable RBAs determined in cell free and whole cell assays point to unhindered drug transport into tumor cells. However, the authors observed reduced RBAs in the whole cell experiment for **23a** (RBA = 0.6%), **23b** (RBA = 0.1%) and **23d** (RBA = 0.15%) indicating a delayed drug permeation through the cell membrane and/or an active drug transport out of the cell. The agents **23a** and **23b** were also comparatively tested on an ER^+ (MCF-7) and an ER^- (Eνsa-T) breast cancer cell line. At a concentration of 5.0 μM **23b** strongly inhibited the growth of ER^+ MCF-7 cells (90% inhibition) despite of its small ER affinity observed in the whole cell assay. The inhibitory effect of **23b** could be reduced by E_2. In accordance with this, the activity of **23b** on the ER^- Eνsa-T breast cancer cell line proved to be markedly lower (44% inhibition). Presumably the specific activity of **23b** against ER^+ breast cancer cells is caused by a receptor-mediated increased insertion into DNA, which must be confirmed with pure cis-$PtCl_2$–platinum(II) derivative. In contrast to **23b** its isomer **23a** was only marginally active at a concentration of 5.0 μM on the two cell lines. Test data of **23d** on ER^+ and ER^- breast cancer cell lines are missing in the publication.



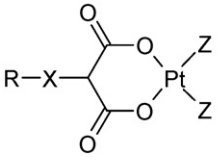


L1

L1	R1	R2	R3	R4
a	NH_2	H	H	OH
b	H	NH_2	H	OH
c	H	H	NH_2	OH
d	H	H	H	NH_2

L2: 4-methylpyridine (py^4) or 2,4,6-trimethyl pyridine ($py^{2,4,6}$)

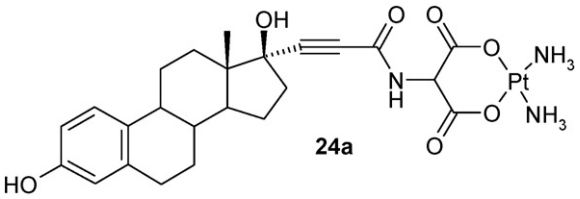
Gandolfi et al. [127,128] studied a series of malonato platinum complexes **24**, in which the steroids were attached directly or via various spacers to the leaving groups.



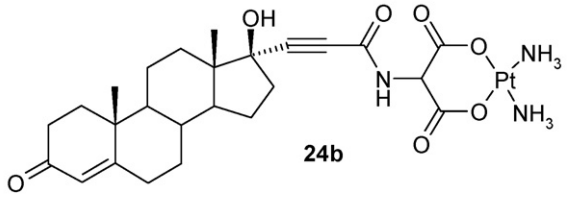
24

Z = NH_3, amine or diamine
X = spacer, see **24a** and **24b**; withouth spacer **24c**
R = steroid

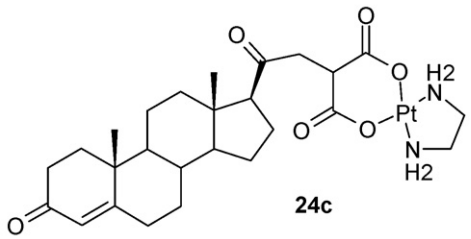
In the following one example each for a Pt-complex linked to E_2 (**24a**), testosterone (**24b**) and progesterone (**24c**) are listed. Data on receptor affinity, anti-breast and anti-prostate cancer activity were not reported.



24a

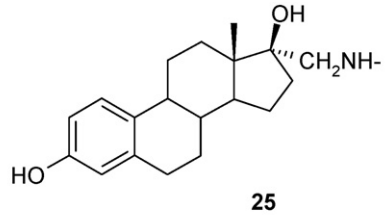


24b



24c

Altman et al. [119] transformed 17α-aminomethylestradiol (**25**) into amides with activated, $PtCl_2$ protected amino acids and peptides to yield cis-$PtCl_2$-complexes like **25a–c**.

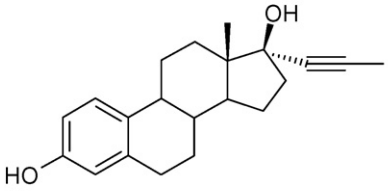


25

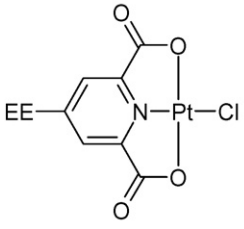
$Cl_2Pt(GlyNHR)_2$ (25a; NHR= 25) and $Cl_2Pt(Gly_nNHR)_2$ (25b and 25c; NHR= 25; n= 2 and 3)

Cis-25a, which was selected for preliminary comparative tests on the ER^+ MCF-7 and the ER^- MDA-MB-231 breast cancer cell line, proved to be marginally active at a concentration of 1.0 μM (7.1% and 14.0% inhibition).

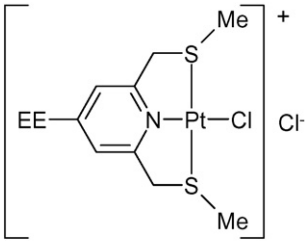
Jackson et al. [129] prepared the monochloroplatinum(II) complexes **26** and **27** (and other metal complexes), in which 17α-ethinylestradiol (EE) was attached to the tridendate ligands pyridine-2,6-dicarboxylate and 2,6-bis[(methylthio)methyl]pyridine. The complexes **26** and **27** exhibited effective binding to the ER (IC_{50} of **26** = 137 nM (RBA = 1.08%), **27** = 39 nM (RBA = 3.3%), DES = 0.2 nM (control)), which was, however, 2 to 3 orders of magnitude weaker than that of DES. In the whole cell assay (ER^+ MCF-7) the ER affinity of **26** was unaltered (IC_{50} = 140 nM) and of **27** significantly higher (IC_{50} = 11.1 nM) than the corresponding value determined in the cell-free system. In the latter experiment compound **27** showed a somewhat higher ER affinity (IC_{50} of **27** = 11.1 nM (15.5 nM)) compared to its corresponding neutral, metal-free ligand (value in parentheses) despite of its mono cationic nature. The increase of the ER binding affinity of **27** possibly arises from an active transport into the cell. The comparative testing on ER^+ and ER^- breast cancer cell lines and studies on the platination of DNA, which can confirm the realization of the drug targeting concept in **26** and **27**, are still missing. Furthermore, it must be remembered that the Pt-pharmacophore in **27** can only form monofunctional but not detrimental bifunctional adducts with DNA.



EE



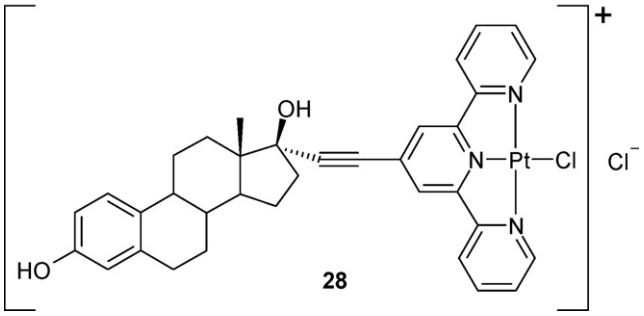
26



27

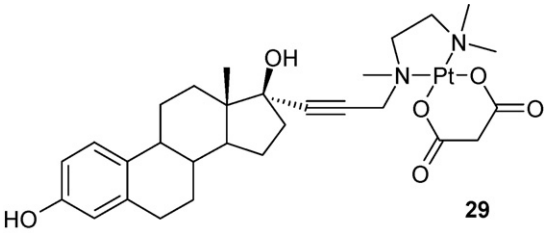
Recently, Hannon et al. [130] synthesized a further ER-affinic Pt-complex by attachment of the ligand ethynylterpyridine (Etpy) to the 17α-position of E_2 and subsequent transformation into [17α-[4′-ethynyl-2,2′:6′,2″-terpyridine]-17β-estradiol]chloroplatinum(II) chloride (PtEEtpy, **28**). X-ray diffraction studies of the ligand **28** confirmed that linkage of the Pt-pharmacophore (PtEtpy) to the 17α-position ensured an orientation below the hydrophobic scaffold of E_2 pointing away from the two OH groups and the upper face of the steroid, which are important for receptor binding. The whole cell ER binding assay on ER^+ MCF-7 breast cancer cells revealed that PtEEtpy (**28**) was transported into these tumor cells and was bound to the ER. However, its ER affinity proved to be by three orders of magnitude smaller than that of DES (IC_{50} values: PtEEtpy = 500 nM; DES = 0.6 nM). Nevertheless, a contribution of the registered though weak ER binding to the cytotoxic potency of PtEEtpy (via drug accumulation) is conceivable. The application of **28** presumably occurs in a concentration range of 0.1 to 10.0 μM, in which Pt-complexes are normally antiproliferative [131]. Unfortunately, a comparative testing of PtEEtpy (**28**) on ER^+ and ER^- breast cancer cell lines estimating the contribution of the ER to the cytotoxicity was not performed. Instead of this it was shown that PtEEtpy (**28**) monofunctionally bound to DNA, presumably to the N^7 of guanine in the major groove. FTICR mass spectra confirmed a linkage through co-ordination to Pt with displacement of the chloride ligand. It must be remembered that marked anti-tumor effects are only observed in Pt-complexes with

bifunctional reaction mode [131]. A covalent binding of PtEEtpy to albumin, which is supposed to function as transport protein for steroids, is also observed.



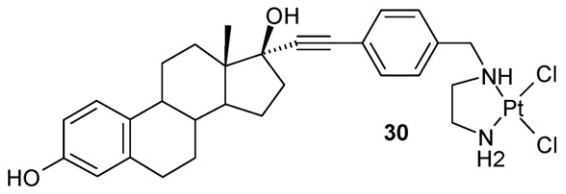
28

Recently, Cassino et al. [132] coupled ethinylestradiol with ethylenediamine derivatives and transformed the resulting compounds to malonato-platinum(II) complexes like **29**. However, the new complexes were incapable of entering the hydrophobic pocket of ER_α and ER_β, as revealed by their RBAs <1%.



29

The same authors described the synthesis of two further Pt-derivatives of 17α-ethynylestradiol, in which the steroid is linked via its ethynyl residue to the Pt-pharmacophores [N-benzylethylenediamine]dichloroplatinum(II) (**30**) or diammine[benzoylaminomalonato]platinum(II) (**31**) [133]. Their binding affinity to the ER amounted to about 2% (E_2: RBA = 100%). In the test on ER^+ MCF-7 and ER^- MDA-MB-231 **30** and **31**, used in a concentration of 1 μM, proved to be inactive or even stimulated the proliferation of the ER^- cell line. The authors suppose that the inherent proliferation stimulating potency of **30** and **31**, detected in tests on the ER^+ MCF-7 breast cancer cell line, overrides the expected cytotoxic effect of these compounds. They argue that ER-agonistic effects usually take place at nanomolar concentration levels, while cytotoxic effects of Pt-complexes are observed at a concentration range of 1-10 μM. Furthermore, they discussed the bio-stoichiometry of the ER-mediated cellular uptake of their Pt-complexes and the subsequent binding to DNA in relation to the findings with cDDP. They concluded that the receptor system was incapable of improving the cytotoxicity.



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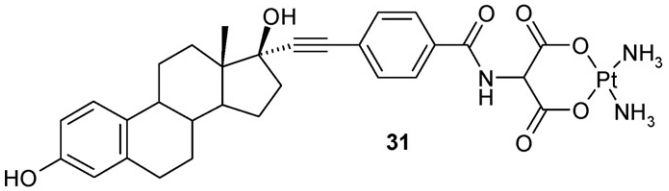
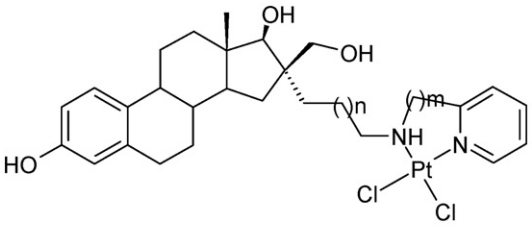


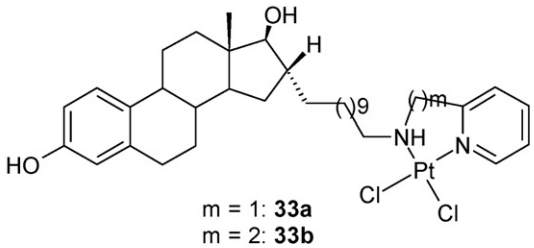
31

In context with 17α-ethynylestradiol Pt-complexes earlier works of Jaouen et al. must be considered, which showed that organometallic complexes linked to E_2 via a 17α-standing ethynyl chain could be effectively applied as potential radiopharmaceuticals for evaluation of the ER status in breast cancer patients [114b,c].

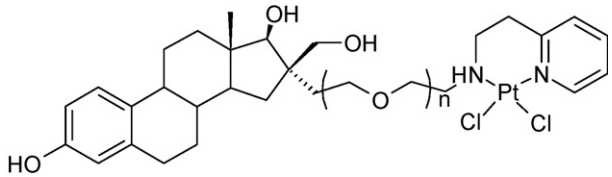
Berubé et al. [134a] reported on the synthesis of a series of compounds (**32**) in which E_2 bearing a 16β-hydroxymethyl group is linked to 2-alkylaminopyridine via an alkyl chain at position 16α and coordinated to dichloroplatinum(II). One of these complexes ($n = 8$, $m = 2$) exhibited a three- to four-times higher cytotoxicity than cDDP on ER^+ and ER^- human breast cancer cell lines. However, the inhibitory effects on the ER^+ lines were not significantly higher than those on the ER^- lines, other than expected for an ER-mediated enrichment in tumor cells.



m = 1 or 2; n = 2, 4, 6 or 8 **32**



m = 1: **33a**
m = 2: **33b**



n = 1 to 5: **34a - e**

Recently, Berubé et al. [134b] described the synthesis and activity of two new complexes **33a** and **33b** in which E_2 is linked at position 16 via an alkyl chain to two different platinum chelates. The novel complexes proved to be highly and equally cytotoxic against ER^+ MCF-7 and ER^- MDA-MB 231 breast cancer cells. Especially [16α,β-[11′-(2″-pyridylethylamino)undecanyl]-1,3,5(10)-estratrien-3,17β-diol]dichloroplatinum(II) (**33b**) – a mixture of epimers at position 16 – was much more cytotoxic than tamoxifen or cDDP. The data do not support the validity of the drug target concept for **33b**. In a binding assay, the ERα affinity of **33b** was equal to that of E_2. However, the ERα- and ER_β-agonistic potencies of **33b**, which have a strong impact on the ER^+ breast cancer growth in vivo (see Section 3 of this review), are unknown.

Further optimization was expected due to the exchange of the carbon spacer by a polyethylene glycol (PEG) chain [135]. The biological activity of these derivatives at ER^+ MCF-7 and ER^- MDA-

MB-231 cell lines, however, is lower than that of the hybrids with carbon spacer. The most promising compound **34e** contains 5 ethylene glycol units and was equipotent to cDDP. Molecular modelling with **34e** revealed an orientation in the LBD of ER_α with the platinum core out side of the binding pocket, which could account for its cytocidal activity.

Further chemical and pharmacological studies are necessary to evaluate the therapeutical value of these interesting drugs. Especially the PEG hybrids could have interesting in vivo biological potential due to their enhanced solubility.

6. Hypothetical mechanism of the anti-breast and anti-prostate cancer activity of ER-affinic Pt-complexes

6.1. Anti-breast cancer activity

In Pt-complexes containing an ER-agonistic or partially antagonistic acting moiety in addition to the DNA chelating Pt-fragment, both pharmacophores contribute to the anti-breast cancer activity by triggering apoptosis (i.e. "programmed cell death") and/or by inhibiting the mutual growth stimulation of breast cancer cells and macrophages/granulocytes.

6.1.1. Triggering apoptosis

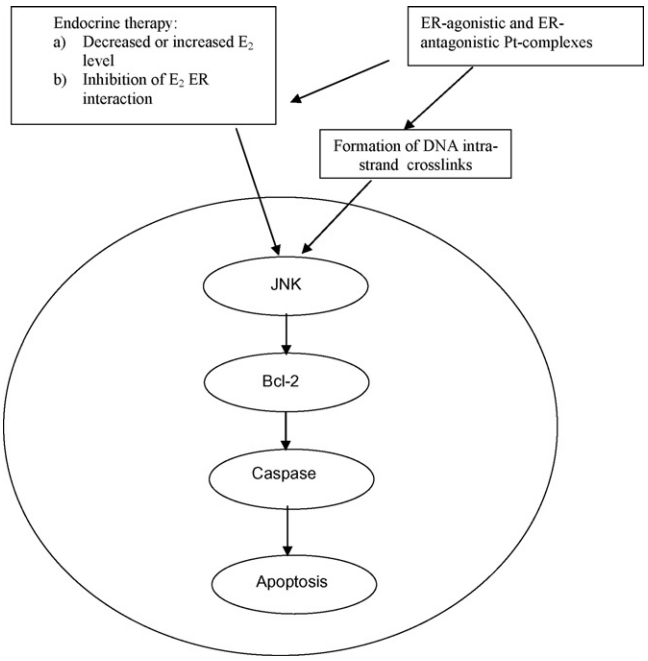
It is supposed that in female rodents bearing hormone-sensitive breast cancer (a) pronounced reduction of the endogenous estrogen level by surgical or medical castration, (b) inhibition of the interaction of endogenous estrogens with their receptors by antiestrogens or (c) administration of a high dosed estrogen cause a change in the balance of cellular proliferation and apoptosis giving rise to a tumor regression. Such a mode of action, which also applies to Pt-complexes containing an additional ER-agonistic or ER-antagonistic pharmacophore, is supported by studies of Michna and Parczyk [136], Vignon and Rochefort [137], Kyprianou et al. [138] and Johnston et al. [139]. The authors observed that tumor regression after endocrine therapeutic measures was accompanied by a decrease of the mitotic index and an increase of the apoptotic index. Fragmentation of tumor DNA into nucleosomal oligomers and histological appearance of apoptotic bodies were characteristic early events that preceded the reduction of tumor volume.

In hormone-sensitive breast cancer, non-ER-affinic Pt-complexes like cDDP and CBDCA can also trigger apoptosis by reacting with sequences of DNA essential for cell proliferation (see Schertl et al. [140] and refs. [31–39] and [61] cited therein). Presumably this effect contributes as well to the anti-breast cancer activity of ER-agonistic or ER-antagonistic Pt-complexes. Furthermore, ER-affinic Pt-complexes are supposed to lead to an enhanced formation of DNA-intrastrand cross links in ER^+ tumor cells due to their intracellular accumulation resulting in an additional increase of apoptosis.

It must be remembered that Pt-complexes can also interfere with the ovarian estrogen biosynthesis and by this reduce the E_2 levels in female rodents, as shown by Sergejew and Hartmann [141] on the example of cDDP and aqua[meso-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]sulfatoplatinum(II). This additional endocrine therapeutic effect of non-estrogenic Pt-complexes (i.e. medical castration) is part of their anti-breast cancer activity, if they are administered in high dosage [140].

Under consideration of these facts, a hypothetical mode of action of estrogenic and antiestrogenic Pt-complexes can be set up as shown in Scheme 1.

Apoptosis in cells of the hormone-sensitive breast cancer is most probably triggered by estrogenic and antiestrogenic Pt-complexes under co-operation of both pharmacophores via impairment of DNA function and interference with growth processes promoted



Scheme 1. Hypothetical mechanism of the anti-breast cancer activity caused by endocrine therapy and by ER-agonistic or ER-antagonistic Pt-complexes—triggering apoptosis.

by endogenous estrogens. These interventions are followed by an elevated activity of the kinase JNK, a modulator of apoptosis. Its substrates are anti-apoptotic proteins like Bcl-2, whose cell death-preventing function is inactivated by phosphorylation. After inactivation of the anti-apoptotic proteins an increase in caspase activity takes place effecting apoptosis (a detailed description of the mechanism of the apoptosis is given in refs. [6,142–144]).

6.1.2. Inhibition of mutual growth stimulation of breast cancer cells and macrophages/granulocytes

The growth inhibition of ER^+ breast cancer in rodents by ER-agonistic Pt-complexes entails a diminution of granulocytes/macrophages.

In untreated ER^+ breast cancer bearing rodents the number of granulocytes and macrophages is elevated under the influence of the tumor and their function is changed towards a promotion of tumor development. This vicious circle of mutual growth stimulation of breast cancer cells and granulocytes/macrophages is described in detail in refs. [145–149] (see also Scheme 2; further refs to the paradoxical role of the immune system in cancer development see refs [150–153]).

It is assumed that hematopoietic growth factors like GM-CSF, which are secreted directly by tumor cells or indirectly by stroma cells, are involved in the expansion of cells of the granulocytes-macrophages lineage. Cytokines from breast cancer cells, e.g. TNFα, IL-1 and IL-6, presumably trigger the secretion of hematopoietic growth factors in stroma cells. It is supposed that (a) the secretion of growth factors directly stimulating the tumor cell proliferation, (b) the secretion of proteases and angiogenesis factors supporting the growth, invasion and metastasis of tumors, and (c) the suppression of immune defence are responsible for the growth promoting effects of granulocytes/macrophages in breast cancer graft bearing rodents. These effects (a–c) weaken in the course of the apoptotic process due to the shrinking tumor volume. Moreover, estrogenic Pt-complexes also seem to inhibit the secretion of hematopoietic growth factors and of stroma cell stimulating cytokines of breast cancer cells, whereby their

anti-tumor activity is intensified. Experiments which support these mechanisms are described in Section 2.2.3, part II of this review.

Studies which were performed to clear up the function of ER_β in the breast cancer therapy with estrogens (see Section 3.1 in part I of this review) suggest a participation of this receptor in the former described mode of action of ER-affinic Pt-complexes.

6.2. Anti-prostate cancer activity

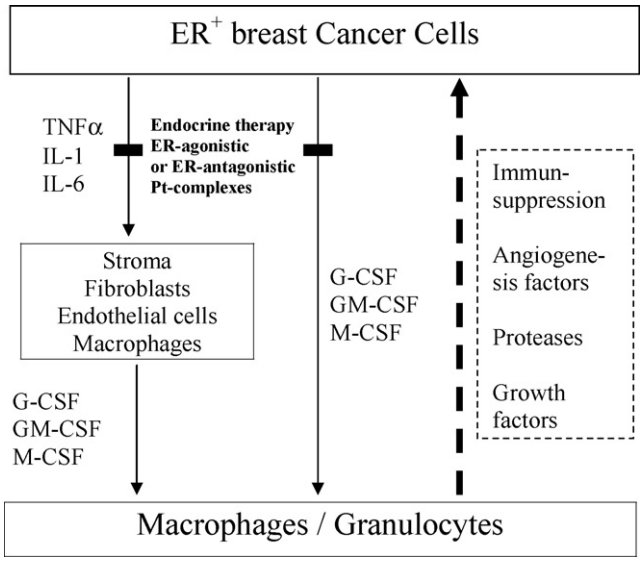
Detailed studies to the mode of action of ER-affinic Pt-complexes, which can significantly inhibit the growth of the AR^+ as well as that of the AR^- PC (see also Section 2.2.4, part II of this review) have not been performed. However, our knowledge, of how estrogens cause their inhibitory activity on prostate cancers (shown in Section 3.2, part I of this review) suggests a mode of action of ER-affinic Pt-complexes, which is comparable to that on breast cancer (see former Section 6.1). Additionally to the processes described in Schemes 1 and 2, in AR^+ PC the estrogenic pharmacophore of Pt-complexes contributes also to their anti-prostate cancer activity: (a) by reduction of the androgen level to castration values [86,90] and (b) by ER_β-mediated AR down-regulation in the cancer cells as shown by Bectic et al. [108] on the example of genistein. Both processes (a and b) trigger apoptosis like the Pt-pharmacophore does (see Kyprianou et al. [138]).

6.3. The need for animal experiments in the screening of ER-affinic Pt-complexes

The cited works suggest an important contribution of the hormonal component to the anti-breast and prostate cancer action of estrogenic Pt-complexes.

Tumor growth inhibiting effects caused by the hormone related mechanisms described in Sections 3 and 6 can at most be partially found in experiments on breast and prostate cancer cell lines usually performed by scientists. Therefore, additional, meaningful in vivo tests, especially on tumor bearing rodents, are indispensable.

In Section 7, a screening system for the discovery of anti-breast and anti-prostate cancer activities of ER-affinic Pt-complexes is presented.



Scheme 2. Hypothetical mechanism of the anti-breast cancer activity caused by endocrine therapy and by ER-agonistic or ER-antagonistic Pt-complexes. Inhibition of mutual growth stimulation of breast cancer cells and macrophages/granulocytes.

7. Screening program

The first step in the in vitro pre-screening of Pt-complexes – described in Table 1, which contain ligands binding to ERα and/or to ERβ, is the determination of their RBAs to each of these two receptors in a competitive binding assay by use of the dextran coated charcoal technique (competitor, $[^3H]$-E_2, RBA of E_2 = 100%) [154,155]. RBAs, which are close to that of E_2 suggest that the corresponding Pt-complexes accumulate in breast (ER_α/ER_β) or prostate cancer cells (ER_β) giving rise to an intensification of the anti-tumor activity caused by the Pt-pharmacophore.

Comparative testing on ER^+ and ER^- breast cancer cell lines (e.g. $ER_\alpha{}^+$ MCF-7, and $ER_\alpha{}^-$ MDA-MB-231), the second step of the pre-screening, is performed to assess the contribution of the hormonal pharmacophore to the growth inhibiting potency in Pt-complexes. However, an increase in activity cannot only be caused by a receptor-mediated elevated intracellular drug level, but also by a displacement of proliferation-stimulating endogenous estrogens from their receptors by an ER_α-antagonistic acting hormonal pharmacophore or by ER_β-agonistic acting hormonal pharmacophores causing proliferation inhibiting and differentiation enhancing effects. In contrast to this, ER_α-agonistic ligands in Pt-complexes stimulate the proliferation of breast cancer cells and therefore weaken the inhibitory activity of the Pt-pharmacophore (compare Section 3.1, part I of this review and test methods see refs. [156,157]). Despite of these facts, most scientists falsely consider a stronger inhibitory effect on $ER_\alpha{}^+$ breast cancer cell lines as proof for a realization of the "drug targeting concept" in the studied Pt-complexes.

A comparative testing on AR^+ and AR^- PC cell lines to verify the "drug targeting concept" in ER_β-affinic Pt-complexes is unnecessary, since both lines contain ER_β (compare Section 3.2, part I of this review).

Cell culture experiments do not correctly reflect the effects of ER-affinic Pt-complexes taking place under in vivo conditions. The reason is that several metabolic processes, which are of importance for the growth of breast and prostate cancer, are localized outside of the tumor cells in the complex network of the body. An inhibition of such processes can be caused for instance by the following events: (a) By ablation of tumor growth stimulating endogenous estrogen ($ER\alpha^+$ MC) or androgen (AR^+ PC); (b) by down regulation of the corresponding receptor system; (c) by impairment of the detrimental function of macrophages and granulocytes in the development of breast and prostate cancer caused by triggering of angiogenic processes. Therefore, comparative in vivo testing on rodent hormone-sensitive and -non-sensitive breast and prostate cancer models is more suitable than that on cell cultures for the assessment of the contribution of hormonal pharmacophores to the anti-tumor activity of Pt-complexes (compare Section 6, part I of this review and ref. [49]).

It is of importance to learn in the pre-screening, if and to which extent ER-affinic Pt-complexes are endowed with ER_α/ER_β-agonistic and/or ERα-antagonistic properties, which contribute to their anti-breast cancer activity (step III). The $ER_\alpha{}^+$ MCF-7-2a breast cancer cell line, stably transfected with the plasmid $ERE_{wtc}luc$, is a useful test model for the detection of ER_α-agonistic and ER_α-antagonistic effects [158]. ER_β-agonistic effects, which are also involved in the activities of ER-affinic Pt-complexes on prostate cancer, can be detected in the test on U-2 OS cells transiently transfected with plasmids encoding for ER_β (pSG5-ER_β FL) as well as the reporter plasmid $(ERE)_2luc^+$ [159] (step IV).

The anti-tumor activity as well as the bioavailability of Pt-complexes is strongly influenced by their capability to react with bionucleophiles. It is widely accepted that the formation of DNA intrastrand cross links, especially of [Pt{d(GpG)}] and [Pt{d(ApG)}], is responsible for the inhibition of tumor growth. The reaction of the

Table 1
Screening program for detection of anti-breast and -prostate cancer activity of ER-affinic Pt-complexes.

In vitro prescreening
I. Estrogen receptor affinity (ER_α and ER_β)
II. Comparative testing on ER_α-positive and ER_α-negative breast cancer cell lines
III. Detection of ER_α-agonistic and ER_α-antagonistic properties on the ER^+ MCF-7-2a cell line, transfected with the plasmid ERE_{wtc}luc
IV. Detection of ER_β-agonistic properties on the U-2 OS cell line, transfected with the plasmid ERE_{wtc}luc

V. Bioavailability
(a) Reaction kinetics under physiological conditions by use of iodide as nucleophile and cisplatin and carboplatin for comparison
(b) Search for an appropriate "leaving group" and development of a galenical formulation for the parenteral administration
(c) Inactivation kinetics of the new "leaving group" formulation by use of albumin as nucleophile, water solubility, testing on breast cancer cell lines
(d) Cellular drug uptake and DNA platination

In vivo testing
I. Influence of promising drugs on the development of the murine breast cancers ER^+ MXT-M-3,2 and ER^- MXT(ovex) in consideration of pharmacokinetics, toxicology and aspects of the mode of action
II Additional investigation of interesting compounds on anti-prostate cancer activity, especially on extension of time to disease progression and growth inhibition of relapsed tumors using Dunning rat prostate cancer models as well as testing on chemopreventive efficacy in the TRAMP mouse model

Pt-complexes with bionucleophiles, especially with human serum albumin (HSA), during their transport to the tumor can result in drug levels, which are too low for an efficient anti-tumor activity. Therefore, it is of interest to know, if the test compound differs from the standard cDDP or CBDCA in its reaction kinetics. This can be studied in a reaction making use of iodide as nucleophile (step Va) [160]. Too fast or too slow reacting compounds cause an insufficient impairment of DNA and require the exchange of their leaving group, by more appropriate ones, especially by such with solubilizing properties or, as far as necessary, the development of a galenical formulation for parenteral administration [161] (step Vb). The inactivation kinetics and water solubility of the new leaving group derivative are investigated to prove that the free drug level is sufficient for optimal anti-tumor activity (step Vc) [160]. Determination of the intracellular and DNA-bound Pt by atomic absorption spectrophotometry reveals, if drug accumulation by breast cancer cells takes place resulting in a high degree of DNA platination (step Vd) [162].

The anti-tumor activity of compounds, which proved to be very promising in the in vitro pre-screening is confirmed by in vivo testing on the murine breast cancer models ER^+ MXT-M-3,2 and ER^- MXT(ovex) [49] as well as on the DMBA-induced breast cancer of the SD-rat [163,164] in consideration of pharmacokinetics, toxicology and aspects of the mode of action (step I).

Pt-complexes with ER-agonistic properties (especially ER_β agonists) are also tested on their activity against rodent prostate cancer models [165–167] with the priorities extension of time to disease progression and growth inhibition of relapsed tumors (compare ref. [90]).

Experiments described in this section are used in part II of this review for pre-clinical testing of non-steroidal Pt-complexes.

8. Conclusion

Studies of numerous scientists show that ER-affinic Pt-complexes are active on estrogen-sensitive tumors like breast and prostate cancer by a complex mode of action in which ER-agonistic and ER-antagonistic properties are involved. ER-mediated accumulation in tumor cells and increased incorporation into their DNA may contribute to the anti-tumor activity. However, the proof is still pending.

In part II of this review studies on platinum complexes with non-steroidal, ER-binding ligands are described.

Acknowledgements

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