

Preclinical and Clinical Studies on the Use of Platinum Complexes for Breast Cancer Treatment

Ingo Ott and Ronald Gust*

Institute of Pharmacy, Free University of Berlin, Königin Luise Str. 2+4, 14195 Berlin, Germany

Abstract: Platinum complexes such as cisplatin and carboplatin are widely used in today's cancer chemotherapy but not in the present therapy of breast cancer, the most frequent epithelial malignancy among women.

As platinum compounds display high antitumoral efficacy against several breast cancer cell lines *in-vitro* they may be an interesting option for future clinical therapy of this disease. On the preclinical stage hormonally active and tissue selective platinum anticancer drugs have been investigated. Clinical trials on established platinum drugs (mainly cisplatin and carboplatin) showed that they can be efficient cytostatics for breast cancer therapy, if patients are carefully selected and suitable combination regimens (e.g. including taxanes) are administered. This review covers the latest findings about new platinum complexes in preclinical studies on the use against breast cancer as well as the outcome of the most relevant clinical trials.

Key Words: Platinum complexes, breast cancer.

INTRODUCTION

Platinum complexes are widely used in today's anticancer therapy based on their ability to covalently bind to the DNA. Cisplatin (**1**) and carboplatin (**2**) (see Fig. 1) are successfully administered in the treatment of epithelial malignancies such as lung, head and neck, ovarian, bladder, and testicular cancer [1]. Oxaliplatin (**3**) is active against many cisplatin resistant tumors such as the widespread colorectal cancer. Other platinum complexes, like e.g. nedaplatin (**4**) and lobaplatin (**5**), are regionally approved in China, Japan and South Korea [2,3].

most frequent cancer among women. As many platinum compounds show high activity against the growth of breast tumor tissue in *in-vitro* and *in-vivo* experiments they could probably be useful therapeutics for this disease and might have been underestimated in this context during the last decades. Comprehensive reviews on the use of platinum complexes against breast cancer have been published in the last years focussing mainly on the outcome of clinical trials [4-6]. However, many platinum compounds are currently investigated in preclinical studies for the use against breast cancer. Many of them combine a DNA binding moiety with a carrier ligand to target selectively mammary carcinoma

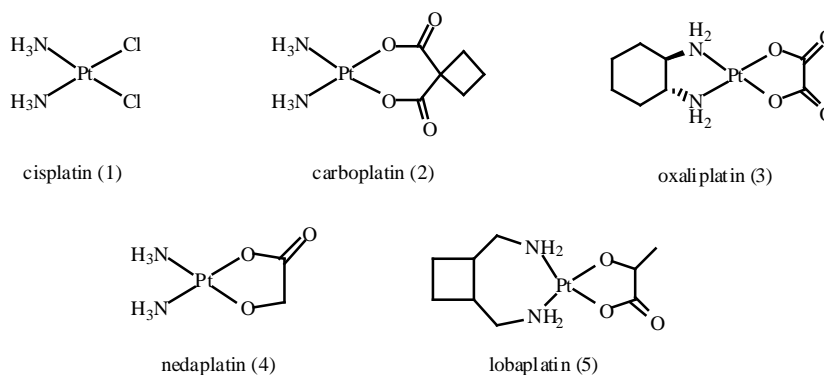


Fig. (1). Examples of clinically approved platinum(II) complexes.

Surprisingly, the platinum complexes did not find their way into the regular therapy of breast cancer, which is the

cells. The estrogen receptor affinity of a carrier ligand will cause a high accumulation of the substances in the tumor cells followed by antitumoral effects triggered by DNA binding *via* the platinum moiety. These properties may lead to well tolerable platinum based anticancer drugs for future use in clinical therapy.

*Address correspondence to this author at the Institute of Pharmacy, Free University of Berlin, Königin Luise Str. 2+4, 14195 Berlin, Germany; Tel: +49 30 838 53272; Fax: +49 30 838 56906; E-mail: rgust@zedat.fu-berlin.de

PARAMETERS TO DESCRIBE THE EFFECTS OF PLATINUM COMPLEXES ON HUMAN BREAST CANCER CELLS

Various *in-vitro* methodologies exist to evaluate the drug targeting concept. Studies on the cytotoxicity and antiproliferative effects of the complexes on cultured breast cancer cells are the most important parameters within the established screening procedures. As the mode of action is based on covalent modification of the DNA the cellular drug uptake and the subsequent binding to the DNA have been studied.

ANTIPROLIFERATIVE / CYTOTOXIC EFFECTS

The most interesting effects of platinum complexes are the antiproliferative / cytotoxic properties of the compounds. A widely accepted form to describe antiproliferative / cytotoxic drug effects is the IC_{50} -value, which is defined as the concentration that reduces the growth or the cell biomass by 50%. Using human breast cancer cell lines IC_{50} -values for the established platinum complexes (cisplatin, carboplatin, oxaliplatin) have been determined in the nanomolar to low micromolar range [7-9]. The values depend on the concrete experimental conditions, like e.g. drug exposure period and number of cells at the beginning of the experiment. In most studies the cells are incubated with the drugs for periods between 24 and 96 hours. Interestingly, for platinum complexes longer exposure periods can lead to more pronounced antiproliferative properties. As shown for cisplatin and carboplatin at MCF-7, MDA-MB 231 and T47D cells incubation of 200 hours and more led to higher cytotoxic effects than incubation up to 100 hours [10].

CELLULAR DRUG UPTAKE AND DNA PLATINATION

The amount of a platinum complex taken up into the cancer cells is an important parameter determining the efficacy of the drugs. Reduced cellular uptake or increased efflux are often associated with drug resistance phenomena.

Cellular uptake studies using atomic absorption spectroscopy or inductively coupled plasma mass spectroscopy showed that cisplatin is continuously taken up into MCF-7 breast cancer cells over up to 48 hours of drug incubation, while the uptake of carboplatin reaches a plateau and the intracellular oxaliplatin concentration begins slightly to decrease after having reached a maximum at approximately 24 hours of exposure [10,11].

The cellular platinum content is best expressed as moles of platinum per mass of cellular protein (e.g. $\mu\text{mol Pt} / \mu\text{g protein}$ - value). Based on this value as well as the mean cellular diameter and mean cellular protein content the intracellular molar concentration can be estimated [10].

The accumulation grade of a drug is the intracellular concentration of the drug divided by the concentration in the extracellular medium. Thus, an accumulation grade of 1 means that intra- and extracellular drug concentrations are the same. Comparative studies showed that cisplatin and oxaliplatin reach accumulation grades of up to 6 in human breast cancer cells, meaning that the intracellular concentrations slightly exceed the extracellular concentrations. For

carboplatin accumulation grades lower than 1 up to 2 were determined. Therefore, carboplatin is taken up into the cells to a significantly lower content compared to cisplatin. Oxaliplatin not markedly exceeds its extracellular concentration. Obviously, the cellular uptake of platinum compounds strongly depends on the nature of the ligands.

The uptake of cisplatin into MCF-7 and T47D human breast cancer cells depends directly on the extracellular concentration and is not saturable, which indicates a passive diffusion mechanism [11,12].

Studies on chromosomal DNA and nuclei isolated from breast cancer cells showed that cisplatin and other platinum complexes migrate into the nuclei and platinate the DNA to the highest levels within the first 6 hours of drug incubation. Roughly estimated only less than 10% of the platinum found inside the cells reaches the nuclei. The amount of platinum binding to the DNA depends on the concentration used in the extracellular medium. Furthermore, correlations with the cytotoxic effects were found [12,13].

HORMONALLY ACTIVE PLATINUM COMPLEXES AND THE CONCEPT OF DRUG TARGETING

One form of breast cancer therapy is based on the fact that certain breast cancers are regarded to be hormone dependent due to an overexpression of estrogen receptors. These can be treated with anti-hormonally active substances, which antagonize the effects of the estrogen receptors (so called anti-estrogens, like e.g. tamoxifen). Attempts to use this concept for the design of new platinum complexes have also been undertaken. The principal goal of these investigations is either to design an anti-hormonally active compound or to use the concept of drug targeting, meaning to deliver the potentially toxic platinum specifically to hormone dependent tissue. In this context the estrogen receptor is regarded as a suitable target as it could be used to translocate potential cytotoxic moieties into the cell nucleus.

Moreover, the strategy is underlined by the findings that estrogen treatment sensitizes MCF-7 cells to cisplatin and carboplatin by triggering the overexpression of HMG-1, a protein which shields the major cisplatin-DNA adducts from nucleotide excision repair [14].

One major strategy to develop platinum complexes with hormonal potency or for drug targeting purposes is to link platinum atoms *via* linkers to established estrogenic or anti-estrogenic substances. Obviously, estradiol and its derivatives or the hormone antagonist tamoxifen are useful candidates for this strategy.

For the evaluation and comparison of new hormonal active compounds the determination of the receptor binding affinity (RBA) value is a useful tool. For the calculation of this parameter the estrogen receptor binding affinity of estradiol (measured by displacement of radioactive labelled estradiol from the receptor) is usually set 100%.

Another parameter is the estrogenic potency of a compound which can be evaluated using breast cancer cells transfected with estrogen receptor response element (ERE) containing transporter plasmids (e.g. ERE_{wTcluc}). Estrogenic active substances trigger the expression of the enzyme lu-

ciferase whose activity can be determined in a chemoluminescence reaction. Antiestrogenic properties can be investigated in competition experiments with estradiol [15].

PLATINUM COMPLEXES WITH LIGANDS DERIVED FROM ESTRADIOL

In an early report Gandolfi *et al.* presented a platinum complex (6) with an estradiol ligand modified in position 3

of the steroid backbone (see Fig. 2) which was comparably cytotoxic to cisplatin in MCF-7 cells [16]. Altman *et al.* prepared platinum complexes of estrone and estradiol with an aminoethoxy group also in the position 3 [17]. One cationic (7) and one neutral (8) complex were investigated for their antiproliferative effects at hormone dependent MCF-7 breast cancer cells. Whereas the neutral complex was inactive within the selected concentration range, the cationic complex exhibited antiproliferative effects in a concentration of 5 μM

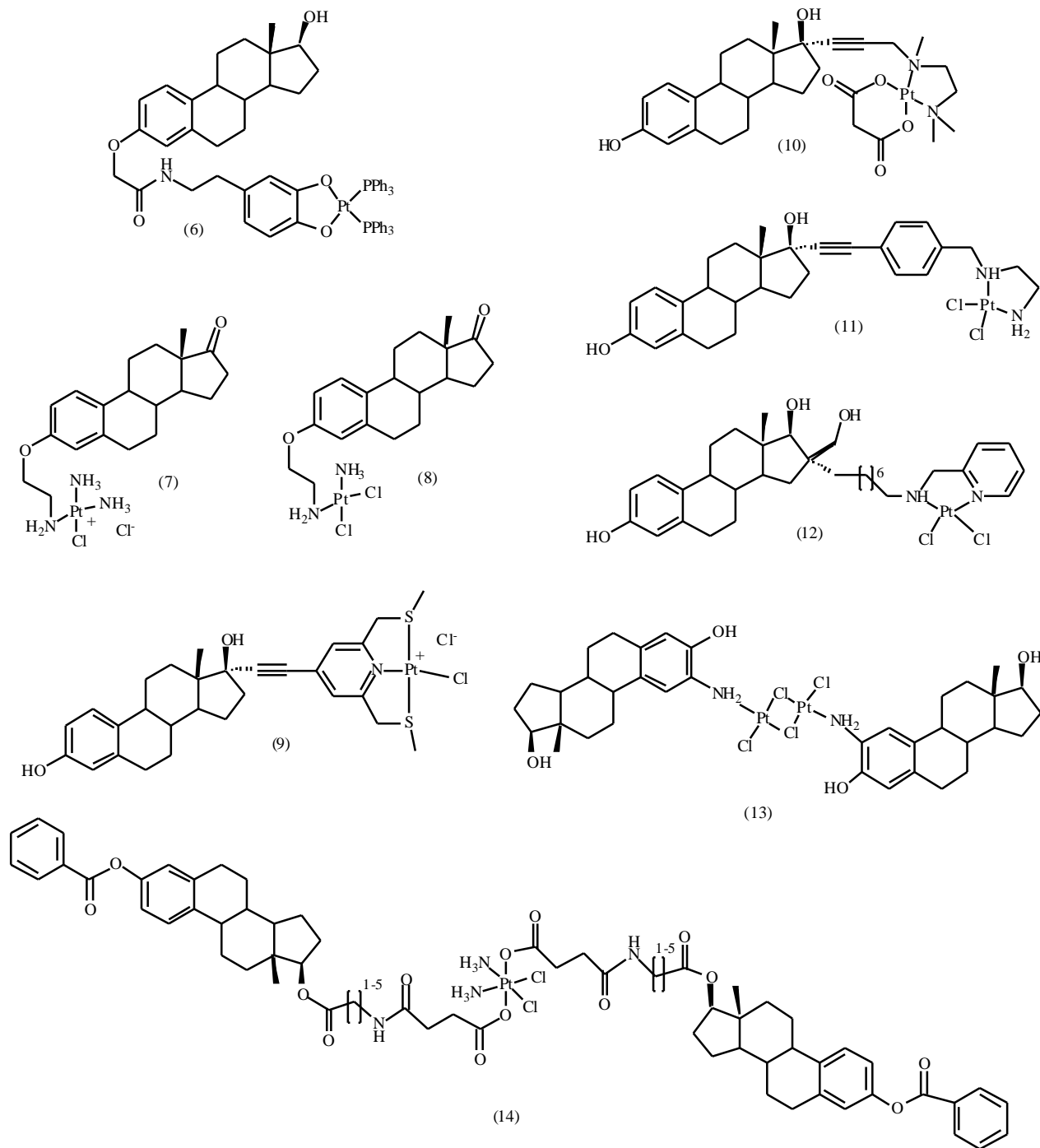


Fig. (2). Platinum complexes with ligands derived from estradiol and estrone.

that were comparable to that caused by tamoxifen. However, the authors report that the compound was also active against leukemia of the mouse, which makes a contribution of hormonal activity to the antiproliferative effects unlikely.

One possible explanation for these results could be seen in the choice of position 3 in the estradiol ligand for attaching the platinum moiety. The replacement of the hydroxyl groups at positions 3 and 17 of the steroid backbone of estradiol is generally detrimental to the receptor interaction as these functions play an important role in the receptor recognition. Spacers linking the platinum to the 17 position, for example, should be by far better tolerated.

Therefore, complexes with ethynylestradiol ligands modified in the 17 position have been prepared [18-20]. Fig. 2 shows some of the compounds exemplarily. While the cationic complex (**9**) achieved an RBA-value of 3.3% the binding affinity of other complexes such as (**10**) was comparably lower. Very recently for other derivatives such as (**11**) a proliferative effect was shown at low concentration indicating estrogenic behaviour of the compounds [20].

The position 16 is also suitable for introducing platinum containing groups to the steroid nucleus, which should not affect the receptor binding of the core too much. Platinum complexes of 17-estradiol (e.g. complex (**12**)) were prepared and investigated for their cytotoxic effects against several human mammary carcinoma cell lines [21-23]. A tissue selectivity between estrogen receptor positive (MCF-7 and ZR-75-1) and negative (MDA-MB 231 and HS578T) breast cancer cells could not be observed for the active compounds. Similar effects were obtained in estrogen receptor positive or negative uterine and ovarian cancer cells.

Interestingly, the length of the alkyl spacer between the steroid backbone and the platinum chelating moiety played an important role. Optimal results were obtained with spacers containing 6 or more carbon atoms. In estrogen receptor binding assays the complexes showed a stronger affinity to the receptor than the natural ligand 17-estradiol. This effect could be attributed to the additional hydrogen bonding ability of the new compounds compared to 17-estradiol and was confirmed by molecular modeling studies.

Amino-estradiol platinum(II) conjugates afforded satisfactorily high RBA-values (up to 14%) and were cytotoxic, however, again without tissue selectivity [24,25]. For one dimeric compound (**13**) an estrogenic effect was postulated as it was able to promote cell growth at low concentrations and suppressed the inhibitory effect of tamoxifen.

For platinum(IV) complexes with two estradiol ligands (**14**) a prodrug concept was proposed [26]. After transport into the cells, reduction of Pt(IV) to Pt(II) and ester hydrolysis, cisplatin and two equivalents of estradiol should be released and trigger pharmacological responses. The estrogen release should thereby cause the overexpression of HMGB1 and thus further sensitize the cells to cisplatin [14]. Immunofluorescence microscopy experiments showed that the complexes were able to induce the overexpression of HMGB1 in MCF-7 cells. However, in cytotoxicity experiments the IC₅₀-values (in the range of 2-5 μM) were essentially the same in estrogen receptor positive (MCF-7) and negative (HCC-1937) breast cancer cells.

PLATINUM COMPLEXES WITH LIGANDS DERIVED FROM TRIARYLETHYLENE (TAMOXIFEN DERIVATIVES)

A series of platinum complexes with triarylethylene ligands were synthesized and biologically investigated by Berube *et al.* (see Fig. 3 (**15**) for an example) [27-30]. In general the cytotoxic effects of these compounds were the more pronounced the longer the spacer between the platinum and the triarylethylene moiety was. The higher RBA-values (approximately 0.2 to 1.5%) were found with the derivatives containing hydroxyl substituents and were in the range of that of the reference compound tamoxifen. Similar to the results obtained with the estradiol derived platinum complexes a tissue selectivity for hormone dependent breast cancer cells could not be achieved.

Top *et al.* prepared leaving group derivatives of tamoxifen containing the diaminocyclohexane (DACH) ligand [31]. A 20/80-mixture of the E and Z isomers of the complex (**16**) depicted in Fig. 3 exhibited satisfactory receptor binding (RBA-value = 6.4%) which was, however, lower than that of the malonate precursor ligand (RBA-value = 20.5%). A derivative without the hydroxyl group in the aromatic ring achieved significantly lower receptor binding (RBA-value = 0.5%). Both compounds proved to trigger antiproliferative effects against MCF-7 cells, which were more pronounced for the hydroxylated compound (IC₅₀-value = 4 μM). Interestingly, both compounds led to antiestrogenic effects in a concentration of 10 μM as determined in MCF-7 cells stably transfected with a reporter gene (pVit-tk-Luc-ERE) allowing the expression of the firefly luciferase under control of an estrogen response element.

HORMONALLY ACTIVE 2-PHENYLINDOLE-LINKED PLATINUM(II) COMPLEXES

The group of von Angerer reported about hormonally active platinum(II) complexes containing phenylindole ligands reaching RBA-values up to 6.5 [32,33]. These agents are structurally related to the antiestrogen zindoxifene (compare structures (**17**) and (**18**) in Fig. 4). Further studies on this class of compounds afforded platinum compounds that were antiproliferative active against hormone dependent MCF-7 cells but not against hormone independent MDA-MB 231 cells. *In-vivo* studies confirmed these data. Growth inhibitory activity was found in the case of estrogen receptor positive MXT mouse mammary tumors but not in the case of the estrogen receptor negative ones. However, endocrine activities as determined in the mouse uterine weight test were low.

DEVELOPMENT OF HORMONALLY ACTIVE [1,2-DIARYLETHYLENEDIAMINE]PLATINUM(II) COMPLEXES

In 1984 Wappes *et al.* reported about [1,2-bis(4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes with an effect on the hormone dependent mammary carcinoma cell line MCF-7 [34]. The neutral ligands were structurally developed from the synthetic estrogen diethylstilbestrol (**19**) and hexestrol (**20**) (compare structures in Fig. 5). The corresponding metal complexes such as (**22**) exhibited lower re-

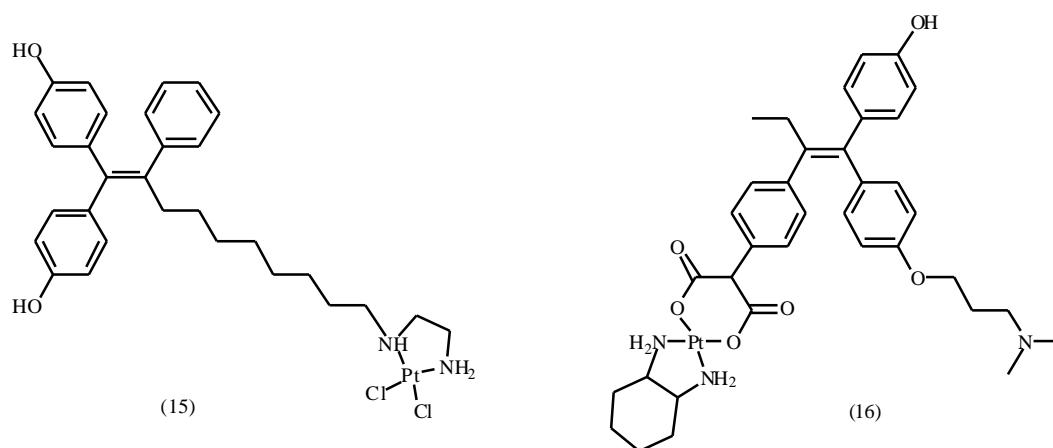


Fig. (3). Platinum(II) complexes with triphenylethylene ligands.

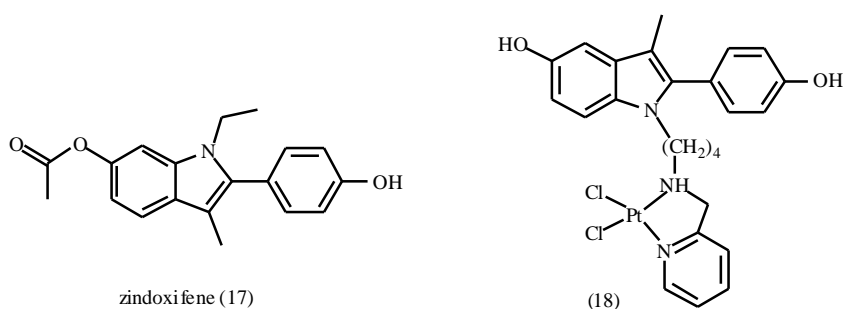


Fig. (4). Zindoxifene and 2-phenylindole-linked platinum(II) complex.

ceptor binding compared to the free ligands e.g. (21). However, some of them reached IC_{50} values lower than $10 \mu\text{M}$ in cytotoxicity experiments with the hormone receptor negative breast cancer cell line MDA-MB 231. With an IC_{50} value of $0.8 \mu\text{M}$ the *S,S*-configured isomer [(*S,S*)-1,2-bis(4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) was the most efficient compound.

Interestingly, this compound also triggered high cytotoxic effects at the hormone dependent MCF-7 cell line in a concentration of $10 \mu\text{M}$, whereas its *R,R*-isomer was significantly less active. Based on the activity in both hormone dependent and independent cell lines, the low estrogen re-

ceptor binding, an additional DNA interaction experiment and structural considerations, the interaction with the DNA became more likely as a mode of drug action for this compound.

Further studies on this class of platinum compounds focussed on the optimization of the substituents in the phenyl rings and the leaving groups at the platinum central atom. Chlorine substituents were introduced into the aromatic rings to enhance the lipophilicity of the target compounds. This strategy led to compounds with mammary tumor growth inhibiting and estrogenic properties [35-37]. Out of a broad series of investigated complexes the meso configured [1,2-

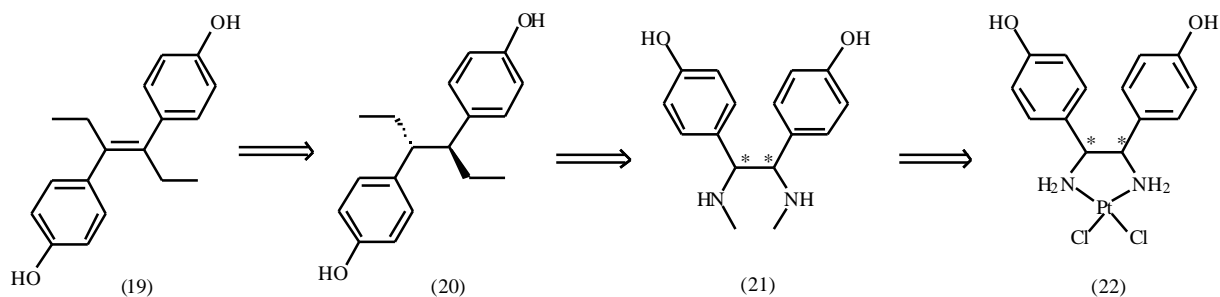


Fig. (5). Development of [1,2-bis(4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes.

bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) (**23**) and its sulfato derivative (**24**) showed especially promising biological activity (see Fig. 6) [38-40].

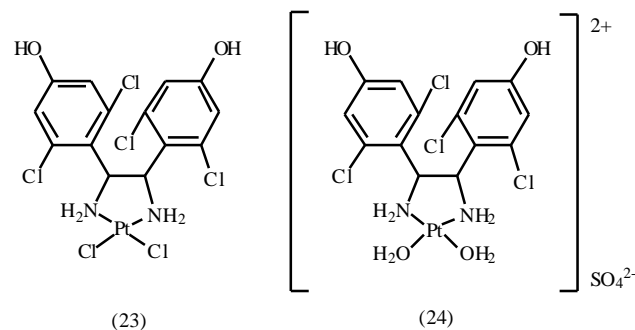


Fig. (6). [1,2-Bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes.

Cytotoxicity experiments revealed that both compounds exhibited IC_{50} values in the 4 - 9 μ M range at the hormone dependent MCF-7 breast cancer cells but were not active at the hormone independent MDA-MB 231 cells. Other structurally related complexes did not show such tissue selectivity. A comparative *in-vivo* study on the ER-positive and ER-negative MXT mammary carcinoma of the mouse further confirmed the preliminary results obtained *in-vitro*. In this assay (**23**) and (**24**) caused high antitumor effects in the ER-positive but not in the ER-negative assay and exhibited a significant uterotrophic effect. Similar results were obtained in DMBA-induced hormone dependent mammary carcinoma of the SD rat. Despite a low receptor binding affinity *in-vitro* these compounds exhibited estrogenic *in-vivo* potency matching the activity of estradiol in the mouse uterine weight test. It is of special interest to note that the water soluble complex (**24**) did not cause kidney damage in contrast to cisplatin. On the estrogen receptor positive MXT mammary carcinoma transplanted in ovariectomized mice a biphasic dose activity curve (meaning at low doses an increase and at high doses a decrease of tumor weight) was observed with (**23**), which was identical with the effect caused by diethylstilbestrol. These results indicate that the described platinum complexes trigger their effects on estrogen receptor positive tissues mainly by their estrogenicity in the same manner as antiestrogens of the diethylstilbestrol type.

Interestingly, (**23**) was also active when administered per os. The effect was superior to subcutaneously given cisplatin at the hormone-sensitive mammary carcinoma of the mouse [41].

Among a series of diastereomeric aqua[1-(2,6-dichloro-4-hydroxyphenyl)-2-phenyl]sulfatoplatinum(II) complexes compounds with threo-configured ligands were more effective cell growth inhibitors than those with erythroligands [42]. They were more effective against MCF-7 cells than MDA-MB 231 cells. *In-vivo* studies using the hormone sensitive murine MXT breast cancer test model were performed in order to find out whether the test compounds acted by their estrogenic potency or by their capability to reduce the endogenous estrogen level. A decrease of tumor and uterine weight was observed for the most active compounds which

indicated a mode of action based on the reduction of the endogenous estrogen level.

Optimization of the alkyl substituents on the nitrogens afforded ligands with relatively high RBA values (up to 20.88%) in the competitive (to estradiol) calf uterus estrogen receptor binding assay but coordination to platinum(II) reduced the affinity to the ER drastically (RBA values 0.1 to 0.5%) [43]. Surprisingly, this did not lead to substantial changes in the estrogenic potency of the substances in the mouse uterine weight test.

Further improvement of the biological effects of this class of platinum compounds could be obtained by the development of sulfato complexes containing fluorosubstituents in the phenyl rings [44,45]. Among these compounds, the racemic diaqua[1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II)sulfate (**25**) (see Fig. 7) was equally active to cisplatin in MCF-7 cells. Platinum measurements revealed that this complex was rapidly and selectively accumulated into the tumor cells within a few hours of drug incubation resulting in a high degree of DNA-platination [46]. The amount of platinum bound to the chromosomal DNA was quantified and followed the order racemic-(**25**) cisplatin \gg meso-(**25**) and correlated well with the results from the cytotoxicity experiments [13].

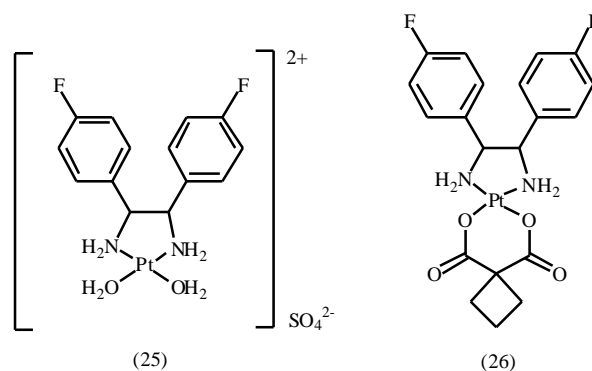


Fig. (7). [1,2-Bis(4-fluorophenyl)ethylenediamine]platinum(II) complexes.

As the [1,2-diarylethylenediamine]platinum(II) complexes are highly reactive species they are subject to inactivation by irreversible binding to nucleophiles (e.g. S-containing bio-nucleophiles like glutathione) [47].

To circumvent this bioavailability problem, complexes with cyclobutane-1,1-dicarboxylic acid (CBDC) ligands were prepared based on the knowledge about the high stability of the CBDC compound carboplatin [10]. Kinetic studies using I⁻ as nucleophile and binding studies to human serum albumin indeed indicated a high stability of this kind of platinum complexes. The racemic derivative of (**26**) was more active towards MCF-7 cells than its meso-configured counterpart and was equipotent with cisplatin. Both compounds were superior compared to carboplatin. Cellular uptake studies showed that racemic (**26**) was distinctly higher accumulated in the tumor cells than cisplatin and carboplatin but less than the racemic sulfato complex (**25**), which in accordance with this, caused higher antiproliferative effects.

[meso-1,2-Bis(2,6-difluoro-4-hydroxyphenyl)ethylenediamine]sulfatoplatinum(II), a complex containing two fluorine substituents in each aromatic ring, proved to be equipotent to cisplatin in the MXT mammary tumor of the mouse. Enzymatic studies indicated a mode of action at the gonadal level by inhibition of the expression of steroidogenic enzymes [48].

The 3-hydroxy derivatives of this promising compound meso and racemic aqua[1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]sulfatoplatinum(II) proved to be highly active on hormone sensitive breast cancer of the mouse but lacked significant activity *in-vitro* on cultured tumor cells [49]. As shown exemplarily for the meso derivative the *in-vivo* effect in the animals is based on a reduction of the endogenous estrogen level caused by an interference with the ovarian steroid biosynthesis. According to this a reversal of the breast cancer inhibiting effect could be achieved by simultaneous administration of estrone. Comparative studies with cisplatin indicated that the minor antiproliferative *in-vitro* activity is probably not due to a rapid inactivation of the compounds by bionucleophiles but rather to an insufficient accumulation in the cultured tumor cells and a steric hindrance of its 2,6-standing fluorine atoms during the interaction with the DNA [50]. N-ethylated derivatives of these compounds did not display strong estrogenic properties indicating that variation of the type and position of the ring substituents is the better choice for optimization of the pharmacological properties [51].

The above described findings altogether show that complexes of the [1,2-diarylethylenediamine]platinum(II) type are useful drugs for the treatment of breast cancer as they can act hormonally *in-vivo* despite a low estrogen receptor binding ability observed *in-vitro*. The *in-vivo* effects seem to be mainly caused by an estrogenic activity causing an uterotrophic effect or by a reduction of the endogenous estrogen level. A cisplatin-like DNA interaction seems to be minor relevant for most of these compounds.

In this context, it is of interest to note that estrogen-like activity of several non-platinum metals has also been reported in MCF-7 breast cancer cells [52,53]. In a similar manner to estradiol, divalent metals (e.g. cobalt, copper) stimulated the cell growth and altered gene expression. Mutational experiments suggested that the metals activated the ER-negative by the formation of a complex within the hormone binding domain of the receptor. Further ongoing studies on cadmium revealed that the effects could also be obtained *in-vivo* [54]. In female Sprague-Dawley rats, cadmium exposure increased the uterine net weight, promoted growth and development of the mammary glands and induced hormone regulated genes in ovariectomized animals.

EFFECTS OF PLATINUM COMPLEXES AND OTHER AGENTS IN COMBINATION ON HUMAN BREAST CANCER CELLS

An emerging number of clinical studies suggest the use of platinum complexes, not only as single agents, but also in combination with other compounds. This interest is further promoted by the occurrence of drug resistance phenomena which trigger a high demand not only for new drugs but also for new therapeutic drug combination regimens. Many of the

clinically observed effects with agents in combination could be predicted or confirmed by *in-vitro* assays using human cell cultures. In this context a synergistic interaction between the drugs is highly desirable. Synergy is defined as the ability of a combination of two or more drugs to achieve a therapeutic effect greater than that expected by the simple addition (additive effect) of the effects of the components used as single drugs.

A promising approach is the combination of platinum complexes with taxanes. Taxanes are products originally isolated from *taxus brevifolia*. Their mode of action is based on the interference with mitosis making them good candidates for use in combination with drugs that act by a different mechanism [55,56].

As observed in T47D cells the combination of cisplatin and taxol indeed caused a higher inhibition of cell growth than both substances alone [57]. In MCF-7 cells synergistic or subadditive effects were reported for the combination of taxol with cisplatin analogues containing sulfur ligands. The combination led to an increased arrest of the cells in the G0G1 phase [58]. The combination of paclitaxel with cisplatin was found to be by far more active than the single agents alone in MDA-MB 231 cells [59]. For the combination of carboplatin with paclitaxel synergistic (MCF-7 cells) or additive (MDA-MB 231, SK-BR-3 cells) interactions were reported [60].

The HER-2/neu gene encodes a transmembrane glycoprotein with intrinsic tyrosine kinase activity (p185^{HER-2/neu}). In approximately 25-30% of the carcinomas of the breast amplification and/or overexpression of this protein was observed and is associated with a poor prognosis and poor clinical outcome [61,62]. Antibodies to the HER-2/neu receptor have been reported to cause cytostatic effects in cells overexpressing p185^{HER-2/neu}. Combination of cisplatin with the antibody trastuzumab (herceptin) yielded a synergistic decrease of cell growth *in-vitro* and complete remissions in experimental animals bearing HER-2/neu-overexpressing human breast cancer xenografts [63,64]. Studies on human breast cancer cells indicated that an interference with DNA repair pathways might be responsible for the observed effects [65,66].

Carboplatin also showed synergistic interactions with trastuzumab in four HER2-overexpressing breast cancer cell lines (SK-BR-3, BT-474, MDA-MB 361, MDA-MB 453) [67].

The triple combination of trastuzumab with cisplatin or carboplatin and the nucleoside analogue gemcitabine triggered synergistic effects in HER-2-overexpressing SK-BR-3 and BT-474 cells [68]. However, the combination of cisplatin or carboplatin with gemcitabine alone also showed synergism.

The enzyme protein farnesyltransferase catalyzes the first step in the posttranslational modification of a number of polypeptides like e.g. ras. In MCF-7 cells the combination of the farnesyltransferase inhibitor SCH66336 with cisplatin produced less than additive effects whereas it triggered additive or synergistic effects in non-small lung cancer and human glioblastoma cells [69].

Oxaliplatin exhibited synergistic or additive antiproliferative activity in different types of breast cancer cells as well as in a hormone independent mammary carcinoma animal model when added to the thymidylate synthase inhibitors 5-FU or AG337 [70].

Finally, for combinations of platinum complexes (including cisplatin) with interferons, additive effects in both MCF-7 and MDA-MB 231 cells have been reported [71,72].

CLINICAL STUDIES

Despite their high potency *in-vitro* platinum complexes are not used in today's regular chemotherapy of breast cancer. This may be caused by disappointing initial findings in early studies on the use of cisplatin in pretreated patients. However, as clearly shown in the following sections under critical patient selection and in combination with the "right partner" they can be valuable agents for therapy.

Hormonally active platinum compounds and complexes using the concept of drug targeting as described in the above sections have not been investigated in clinical trials up to now. Based on the promising properties found in the pre-clinical stage these agents might be considered in future phase I and II trials.

CURRENT THERAPY OF BREAST CANCER

Besides surgery and radiotherapy, chemotherapy plays an important role in today's treatment of breast cancer. There are two options available for the chemotherapy of mammary carcinoma: the classical chemotherapy using cytostatics and the hormonal therapy.

In the classical chemotherapy of breast cancer taxanes and anthracyclines are the most active agents. Paclitaxel or vinorelbine are often used as single agents. In the most cases a combination of two or more cytostatics is administered. Established therapy regimens include CMF (cyclophosphamide, methotrexate, 5-fluorouracil), AC (doxorubicin, cyclophosphamide), EC (epirubicin, cyclophosphamide) or FAC (5-fluorouracil, doxorubicin, cyclophosphamide) [73].

The hormonal therapy makes use of the fact that many breast tumors depend on the presence of estrogen. Thus, anti-estrogens like e.g. the stilbene analogue tamoxifen can be useful in the therapy of ER-positive tumors. The use of such hormonal antagonists marks the first step towards the development of tissue selective cytostatics.

Platinum complexes are not established in the current therapy of mammary tumors yet.

PLATINUM COMPLEXES IN BREAST CANCER MONOTHERAPY

Platinum complexes were initially evaluated against breast cancer in monotherapy. The majority of these studies showed that they have a minor activity if used in pretreated (resistant) patients but a significantly higher potency if used in first-line therapy.

SINGLE AGENT CISPLATIN

An impressive overall response of 54% was reported for cisplatin used as first-line single drug in metastatic breast

cancer. Among the 35 patients, who had not received prior chemotherapy, 13 showed complete remissions [74]. These results were further confirmed by another study using 30 mg/m²/d for four days every 3 weeks on 20 previously untreated patients [75]. Nine partial responses in 19 evaluable patients (one patient refused further therapy following a single day of cisplatin) but no complete remissions were observed, this corresponding to a 47% response rate. Three patients developed serum creatinine levels above 2.0 mg/dL, which made a dose reduction or discontinuation of the treatment necessary.

These promising results are in strong disagreement with studies, in which cisplatin was given as single agent to patients, who had received prior chemotherapy. In these studies the overall response was lower than 11% [4,5]. This effect may be due to the development of drug resistance during the initial therapy.

For example, Martino *et al.* treated 36 women with metastatic breast cancer refractory to conventional therapies with two dose schedules (15 mg/m² day 1 to day 5 repeated at 28-day intervals or 100-120 mg/m² as single doses in 28-day intervals) [76]. No response was recorded in the 15 mg/m² group and 2 (15%) partial responses in the 120 mg/m² group were observed in the 13 evaluable patients.

According to this fact Yap *et al.* found no therapeutic activity in 26 patients refractory to conventional chemotherapy who had received cisplatin 100 mg/m² i.v. as single dose in 3-4 week intervals or as continuous intravenous infusion (20 mg/m²/day) over a period of 5 days at 4-week intervals [77].

These data indicate that cisplatin may be an active drug in metastatic breast cancer but only if used in first-line therapy.

SINGLE AGENT CARBOPLATIN

Carboplatin used in previously treated patients with metastatic breast cancer showed only weak effect with an overall response rate of 6% [5,6]. Thus, only one of 30 previously treated patients responded to carboplatin at a dose of 450 mg/m² every 5 weeks [78].

Similarly to the results obtained with cisplatin the activity was by far better if carboplatin was administered in patients without prior chemotherapy. Kolaric *et al.* performed a phase II study on 20 untreated metastatic breast cancer patients. Carboplatin was given i.v. at a dose of 400 mg/m² on day 1 with a 3 weeks rest period. Two complete remissions and 2 partial responses were observed (20% response rate) and lasted for 4 months in average. The drug toxicity was moderate with anemia, leukopenia, thrombocytopenia and nausea/vomiting as side effects but it produced pronounced myelotoxicity which limited the number of therapy cycles to 5 [79]. Another study was undertaken using the same dose (400 mg/m²) administered intravenously every 4 weeks in 34 metastatic breast cancer patients who had not been exposed to prior chemotherapy. One patient obtained a complete and 11 patients a partial response, resulting in an overall response rate of 35% (11 of 34 patients). Toxicity again was mild (mainly emesis, leukopenia, and thrombocytopenia) [80].

In another phase II study on advanced breast cancer patients carboplatin was administered using a pharmacokinetically guided dose schedule in which the dose was calculated on the basis of the glomerular filtration rate (GFR) using the Calvert formula with an AUC of 7 mg/mL*min [81]. Anemia (42%), leukopenia (20%), thrombocytopenia (35%) and nausea/vomiting (39%) were the most common observed toxicities. The overall response of this treatment was 25%. One of 13 previously treated patients responded to the therapy as compared with 9 of 27 (33%) patients who had not received previous chemotherapy. Thus, this study confirms the above mentioned reports indicating that carboplatin is moderately active in previously untreated but not in previously treated patients.

SINGLE AGENT OXALIPLATIN

Mathe *et al.* reported about activity of oxaliplatin against breast cancer in phase I studies. Two of 12 patients experienced responses [82,83].

Oxaliplatin was used in a phase II study as single agent in anthracycline pretreated advanced breast cancer patients at a dose of 130 mg/m² on day 1 repeated every three weeks [84]. Three (21%) of 14 patients displayed a partial response. The treatment was well tolerated, no severe toxicities were encountered.

SINGLE AGENT IPROPLATIN

Casper *et al.* treated 25 women with advanced breast cancer in a phase II clinical trial with 275 mg/m² iproplatin (**27**) (see Fig. 8) administered intravenously every 4 weeks [85]. Only 2 patients experienced major therapeutic responses (8%). Both of them had not undergone extensive chemotherapy prior to treatment. Myelosuppression, thrombocytopenia, leukopenia, nausea, vomiting were among the toxic side effects.

Iproplatin was investigated in another study on 35 previously treated patients with advanced breast cancer [86]. The drug was given intravenously in a dose of 45 mg/m² for 5 consecutive days and repeated every 28 days. Among the 29

evaluable patients 1 showed a partial response and 2 presented stable disease. Similar to the trial of Casper *et al.* myelosuppression, thrombocytopenia and neutropenia were the common side effects.

Another study using 240 mg/m² iproplatin every 4 weeks showed only weak activity of the drug with 2 (7%) of 32 pretreated patients responding to the treatment [78].

SINGLE AGENT CI-973

A phase II study on CI-973 (**28**) in 26 patients suffering from refractory advanced breast cancer was performed [87]. CI-973 had shown elevated antitumor activity against murine tumors and also cisplatin-resistant tumors in previous investigations and had been associated with lower side effects in phase I clinical trials [88-90]. In this study CI-973 was administered intravenously over 30 min in a dose of 230 mg/m² as a single dose on day 1 of a 21-day treatment cycle. Only 2 patients (8%) showed partial remissions. However, the patients had received one prior chemotherapy which might have limited the efficacy of CI-973 similar to the results obtained with cisplatin and carboplatin (see above). The hematologic toxicity was severe. Nearly all patients experienced granulocytopenia which made a dose reduction for 15 of the 26 patients necessary. Non-hematologic toxic effects were mild and mainly consisted of nausea, vomiting, fatigue, minimum paresthesia and hypomagnesemia.

SINGLE AGENT ZD0473

A phase II study on ZD0473 (**29**) was performed with 33 advanced or metastatic breast cancer patients [91]. Doses of 120 or 150 mg/m² were given as an i.v. infusion every 3 weeks. The toxicity mainly consisted of thrombocytopenia, neutropenia, fatigue, nausea and vomiting. Disappointingly only 1 patient showed a partial response.

SINGLE AGENT SATRAPLATIN

In a phase I study on the orally given platinum complex satraplatin (**30**) a tumor shrinkage was seen in 2 of 4 breast cancer patients [92]. However, the effect did not meet the

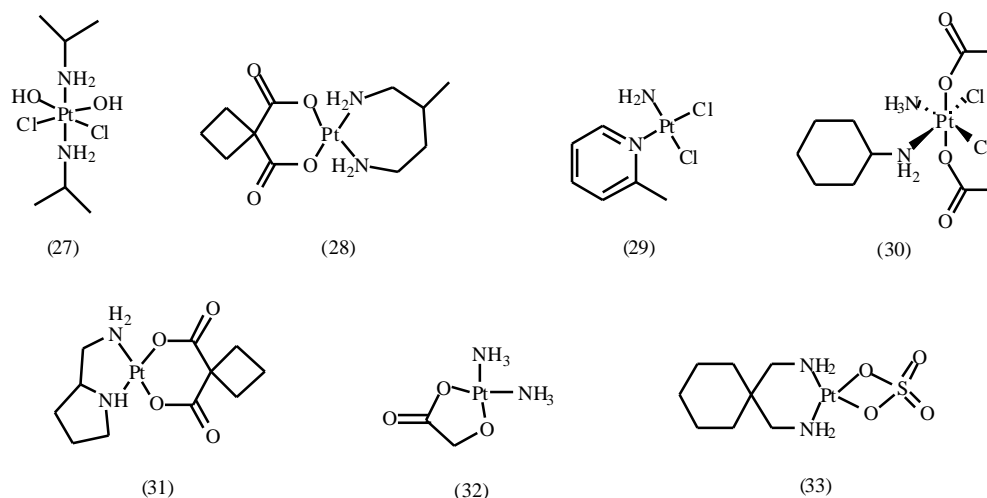


Fig. (8). Structures of platinum complexes investigated in monotherapy.

response criteria. Both responding patients had received prior chemotherapy and were resistant to doxorubicin. The study was performed dose escalating with a daily dosage from 50 to 120 mg/m² for five consecutive days in 23 patients with solid tumors. The maximum tolerated dose was 120 mg/m²/day, the dose limiting toxicities were leukopenia, thrombocytopenia, anemia and diarrhea.

SINGLE AGENT DWA2114R

DWA2114R (**31**) showed activity against breast cancer cell growth *in-vitro*. The effects were comparable to that of carboplatin [9]. DWA2114R was administered at doses of 800-1000 mg/m² every 4 weeks on minimal two cycles and achieved an overall response rate of 21% [93].

SINGLE AGENT NEDAPLATIN

The cisplatin analogue nedaplatin (**32**) achieved a response rate of 13% at a dose of 100 mg/m² [94]. The major toxic effects were hematotoxicity, thrombocytopenia, leukopenia, and gastrointestinal toxicity.

SINGLE AGENT SPIROPLATIN

Spiroplatin (**33**) was investigated in a phase I clinical trial. One of 9 patients showed a complete response. Except the responding patient, all had been treated with cisplatin before and were considered to be resistant to the parent compound. Myelosuppression and renal toxicity were dose-limiting [95].

PLATINUM COMPLEXES IN BREAST CANCER COMBINATION THERAPY

In breast cancer chemotherapy, combination regimens containing two or more drugs are successfully used in the clinic. In preclinical studies, the coadministration of platinum complexes to other cytostatics (e.g. taxanes) led to additive or synergistic effects (see above). A broad variety of combination therapy studies on the use of platinum agents together with other established anticancer agents against breast cancer have been reported.

PLATINUM COMPLEXES AND ETOPOSIDE

Etoposide is a topoisomerase II inhibitor with good oral bioavailability. As a single agent it has only weak activity against metastatic breast cancer [6]. However, animal studies on mice and *in-vitro* studies on spheroid cells showed a synergistic effect with cisplatin [96,97].

CISPLATIN AND ETOPOSIDE

The efficacy of cisplatin plus etoposide was evaluated in breast cancer patients who had failed one previous chemotherapy regimen for advanced disease or had relapsed within 12 months of adjuvant chemotherapy [98]. Etoposide (130 mg/m²/day) was initially given as a continuous infusion for 3 consecutive days and cisplatin (45 mg/m²/day) as a continuous infusion for the latter 48 hours of the etoposide administration. Eleven of the 44 patients showed partial responses (25% response rate). The toxicity of the treatment was severe (e.g. pancytopenia, gastrointestinal upset and renal insuffi-

ciency) and two treatment-related deaths (one sepsis and one renal failure) occurred during this study.

Cisplatin at high dose (100 mg/m²) or low dose (60 mg/m²) was combined with etoposide at a dose of 100 mg/m² as third-line therapy [99]. Independently from the cisplatin dose 12% of the 78 evaluable patients experienced partial responses. In the high dose group, one complete response was observed. However, in this group the toxicity of the treatment was also higher with one toxic death occurring.

Cocconi *et al.* performed a first-line chemotherapy for metastatic breast carcinoma using 100 mg/m² cisplatin (day 1) and 100 mg/m² etoposide (day 1, 3 and 5) every 3 weeks in comparison to a regular CMF treatment [100]. Among the 140 patients, a similar extent of complete remissions (12% in the platinum containing regimen and 11% in the CMF regimen) was seen in both groups but complete plus partial remission rates were somewhat higher in the group of cisplatin treated patients (63% in the platinum containing regimen and 48% in the CMF regimen). However, the hematological toxicity was significantly higher in the cisplatin-etoposide treated group and frequent gastrointestinal side effects were observed.

In a study on 22 patients with metastatic breast carcinoma cisplatin and etoposide were given each at a dose of 100 mg/m² [101]. Objective responses were seen in 50% of the patients. One patient had a complete response.

From these studies, it can be concluded that the combination of cisplatin with etoposide has moderate activity at the price of elevated toxicity.

CARBOPLATIN AND ETOPOSIDE

The combination of carboplatin with etoposide showed, in several studies, better results in previously untreated patients (mean response rate of 29%) than in previously treated patients (mean response rate 16%) [6].

In a phase II study carboplatin and etoposide were used in previously treated and previously untreated patients with metastatic breast cancer [102]. Among 19 patients without prior chemotherapy 1 complete and 7 partial responses were observed (overall response rate 42%) but only one partial response among 26 pretreated patients was observed.

As a first-line treatment for 33 patients, the combination of carboplatin (300 mg/m²) and etoposide (100 mg/m²) resulted in 6% complete and 21% partial responses [103]. The major toxicity was myelosuppression.

In a group of 27 pretreated metastatic breast carcinoma patients who received carboplatin at a dose of 100 mg/m² plus etoposide 100 mg/m², 5 (19%) partial responses were observed [104].

PLATINUM COMPLEXES AND VINOURELBINE

Vinorelbine is a semi-synthetic vinca-alkaloid showing high activity in breast cancer. The medium response rate as a single agent in first-line setting was 43% [6].

CISPLATIN AND VINOURELBINE

Fifty-eight previously treated patients with metastatic breast carcinoma, in whom previous anthracycline therapy

had failed, received cisplatin (20 mg/m²/day) followed by vinorelbine (6 mg, i.v., bolus and then 6 mg/m²/day) on days 1-5 every 21 days. Twenty-four (41%) patients achieved an objective response (including 2 complete responses). Neutropenia was the dose limiting toxicity [105].

In another phase II study on cisplatin plus vinorelbine for heavily pretreated women with metastatic breast cancer doses of 30 mg/m²/day over three days (cisplatin) and 25 mg/m² (vinorelbine) on days 1 and 8 or 15 were administered [106]. The overall response rate was 61% (16 of 23 patients) and 26% of the patients experienced a complete remission.

Based on these studies, it becomes obvious that the combination of cisplatin with vinorelbine is active and well tolerated in heavily pretreated and anthracycline-resistant patients with metastatic breast cancer.

CARBOPLATIN / OXALIPLATIN AND VINOELBINE

In a dose escalating phase I study the combination of carboplatin and vinorelbine showed activity in anthracycline or taxane pretreated metastatic breast cancer patients [107]. The combination was also active in phase II when given as second-line therapy (overall response rate 46%) [108]. Oxaliplatin in combination with vinorelbine was active against breast cancer in a dose escalating phase I study [109].

PLATINUM COMPLEXES AND ANTHRACYCLINES

Anthracyclines are widely used active drugs against breast cancer. Interestingly, only few studies have been performed on the combination with platinum complexes. The overall results of these investigations are disappointing.

CISPLATIN AND ANTHRACYCLINES

Mitoxantrone (7.5-12.0 mg/m²) and cisplatin (100 mg/m²) were given as second-line therapy in 30 patients with advanced breast cancer [110]. The treatment resulted in limited activity (2 partial responses in 29 eligible patients) with considerable toxicity (vomiting, thrombocytopenia, granulocytopenia, leukopenia, anemia).

CARBOPLATIN AND ANTHRACYCLINES

Carboplatin (250 mg/m²) was combined with 4-epidoxirubicin (90 mg/m²) or mitoxantrone (10 mg/m²) [111]. Of the 11 patients only 2 showed stable disease. Hematological toxicity was strong.

IPROPLATIN AND ANTHRACYCLINES

Iproplatin (doses from 150 to 250 mg/m²) was investigated in a phase I clinical trial in combination with doxorubicin (doses 30 to 50 mg/m²) [112]. Nine (35%) of 26 previously untreated patients responded to the therapy. In the group with pretreated patients similar results were observed with 5 (23%) of 22 responding patients. Myelosuppression was the dose-limiting toxicity. The authors did not recommend further investigation of this combination due to the observed toxicities (including nausea, diarrhea and malaise).

PLATINUM COMPLEXES AND NUCLEOSIDE ANALOGUES

Nucleoside analogues are active compounds in the treatment of breast cancer. The fluorinated pyrimidine derivative 5-fluorouracil is commonly used in combination with other agents.

CISPLATIN AND NUCLEOSIDE ANALOGUES

In a study on 44 women with advanced breast cancer, who had all received a previous chemotherapy, only 3 (7%) responded to the combination of cytosin arabinoside (45 mg/m²/day for 3 days) and cisplatin (100 mg/m² on day 2) [113]. This regimen caused significant toxicity with two lethal toxic reactions. Life-threatening leukopenia and thrombocytopenia as well as severe renal toxicity were common side effects.

In another trial 30 pretreated patients with adenocarcinoma of the breast received cisplatin (30 mg/m²) plus gemcitabine (750 or 1000 mg/m²) on days 1 and 8 or on days 1, 8 and 15 of a 21 or 28 days cycle [114]. Three (10%) patients had a complete and 12 (40%) a partial response. Toxicity consisted mainly of neutropenia, anemia and thrombocytopenia.

The combination of 5-fluorouracil (300 mg/m²) with cisplatin (50 mg/m²) was found to be active with an overall response rate of 26% in 77 pretreated patients [115].

CARBOPLATIN AND NUCLEOSIDE ANALOGUES

Carboplatin (60 mg/m²) plus 5-fluorouracil (1000 mg/m²) as second-line therapy in 19 patients afforded only a 5% response rate [116].

OXALIPLATIN AND NUCLEOSIDE ANALOGUES

In a phase II study oxaliplatin (130 mg/m²) and 5-fluorouracil (1000 mg/m²) were given to taxane- and anthracycline-pretreated patients [117]. In 60 patients accessible for response the overall response rate was 27%. Similar results (33% partial responses) were obtained in a study on 12 patients pretreated with both anthracyclines and taxanes [118].

BBR 3464 AND NUCLEOSIDE ANALOGUES

The trinuclear platinum compound BBR3464 (**34**) (see Fig. 9) was investigated in a phase I study in patients with advanced cancer in combination with 5-fluorouracil [119]. Of 14 patients, 3 had stable disease and 1 a partial response. The partial response was observed in a 53-year old female with an invasive carcinoma of the right breast, who had been heavily pretreated.

PLATINUM COMPLEXES AND TAXANES

Taxanes are agents that stabilize microtubules by inhibition of the depolymerisation process and thereby block the proliferation of cancer cells [120,121]. In the last decade the interest in taxanes for the treatment of breast cancer has been rapidly growing. Agents like docetaxel proved to be significantly active in several clinical trials and anthracycline –

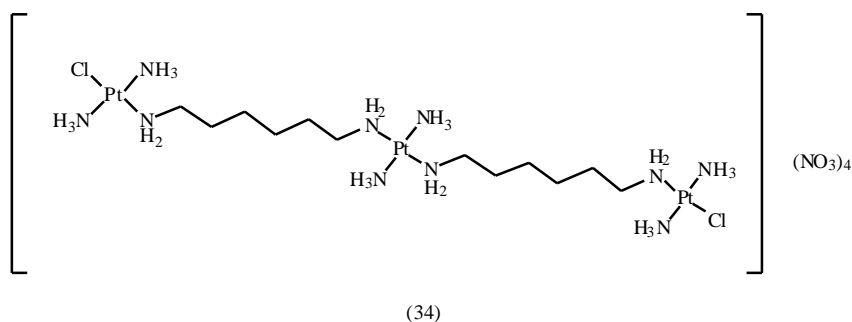


Fig. (9). BBR3464.

taxane combinations also have shown high efficacy. The interest in developing non-anthracycline containing combination regimens further increased the need in research on platinum – taxane combinations. There seem to be no cross-resistances between the two classes of drugs and the drugs do not induce overlapping toxicities with the exception of neurotoxicity [122]. Additionally, *in-vitro* investigations showed that the effects of both drugs are additive (see above).

CISPLATIN AND TAXANES

Forty-three women with metastatic breast cancer were enrolled in a phase II study on cisplatin in combination with paclitaxel [59]. Twenty-seven patients had not received prior chemotherapy for the metastatic disease but of those, 16 had received prior anthracycline-based (FEC), 7 CMF and 4 hormonal adjuvant chemotherapy. All 16 patients pretreated for the metastatic disease had received anthracyclines. The untreated women received paclitaxel weekly for a minimum of 6 weeks in a dose of 85 mg/m² followed by 40 mg/m² of cisplatin while the pretreated women received 75 mg/m² paclitaxel followed by the same dose of cisplatin. Granulocyte colony-stimulating factor (G-CSF) 5 µg/kg was administered subcutaneously as support two times each week for the whole treatment period. Seven complete (26%) and 15 partial responses were observed in the untreated group, corresponding to an overall response rate of 81%. In the pretreated group 6 patients (37%) experienced partial responses. Additionally to these promising results the therapy was quite well tolerated. Hematological toxicity and peripheral neuropathy were more frequent in the pretreated group and the non-hematological toxicity was negligible in the majority of the patients. Only 2 patients of the pretreated group had to discontinue the therapy because of myelosuppression.

Thirty-nine patients with metastatic breast cancer, who were primary or secondary resistant to previous anthracycline treatment, received docetaxel in a dose of 75 mg/m² followed by cisplatin in a dose of 60 mg/m² every 3 weeks for a maximum of 6 weeks or until disease progression [123]. The response rate to this treatment was 31% with 3 complete responses. Neutropenia was the most frequently observed severe hematologic toxicity occurring in 39% of the patients and asthenia and nausea the most common non-hematologic toxicities. Thus, this combination seems to be a relatively safe and effective therapy in patients with anthracycline-resistant metastatic breast cancer.

Gelmon *et al.* treated 29 mainly pretreated women with paclitaxel 90 mg/m² and cisplatin 60 mg/m² biweekly and reported an overall response rate of 85% with 3 patients (11%) obtaining a complete remission [124]. The 3 patients who experienced complete remissions had all been previously treated with anthracycline containing adjuvant regimens. Neutropenia was the dose-limiting toxicity. Fatigue, nausea, and peripheral neuropathy were mild and tolerable.

However, a similar study with the same doses of both agents in 16 patients afforded an overall response rate of only 23% and severe toxicity [125]. Most of the patients in this study had been pretreated with doxorubicin containing adjuvant chemotherapy and had three or more sites of disease. Obviously, the drug combination is not very efficient in patient populations possessing the described characteristics.

Forty-four patients with no more than one chemotherapeutic treatment for advanced disease entered a phase II treatment consisting of 200 mg/m² paclitaxel administered as 24-hour intravenous infusion followed by cisplatin in a dose of 75 mg/m² intravenously and 5 µg/kg G-CSF subcutaneously on day 3 [126]. Among the 42 patients accessible for response 5 (12%) experienced a complete and 17 (41%) a partial response, with an overall response rate of 53%. However, a cumulative neurotoxicity was significant and dose-limiting for this treatment. This could be probably due to the higher dosage used in this study. Other significant toxicities included fatigue, nausea and vomiting.

CARBOPLATIN AND TAXANES

Sixty-six women with advanced breast cancer were enrolled in a first-line phase II chemotherapy [127]. Thirty-nine of them had received adjuvant chemotherapy and 22 of those were treated with an anthracycline or mitoxantrone containing regimen. Paclitaxel was administered at a dose of 175 mg/m² by three-hour infusion followed by carboplatin at an AUC of 6 mg x min/mL every three weeks. Twelve percent complete and 42% partial responses were reported. The most frequent toxicity was granulocytopenia (24%).

In a similar study 200 mg/m² paclitaxel were administered as 3-hour infusion with 6 mg/mL per minute carboplatin every 3 weeks to 53 women with metastatic disease [128]. Prior adjuvant chemotherapy, including anthracycline-based regimens, and prior hormonal therapy were allowed but no prior therapy with platinum or taxanes. Of the 50 evaluable patients 16% had a complete and 46% a partial

response. The therapy was generally well tolerated, the predominant toxicity was neutropenia occurring in 82% of the patients.

Activity and good tolerability was also reported in patients with anthracycline-resistant advanced breast cancer [129]. Thirty-seven patients received paclitaxel at 200 mg/m² by 3-hour infusion and carboplatin at an AUC of 7 mg/mL per minute every 4 weeks with G-CSF support. Five (14%) women showed complete and 11 (30%) partial responses.

In a multicenter trial 100 patients (61 of them had received prior chemotherapy) with advanced breast cancer were treated with paclitaxel (100-135 mg/m²) followed by carboplatin (AUC 2) [130]. The overall response rate among 95 assessable patients was 62% (8% complete and 54% partial responses). Neutropenia and leukopenia were among the most common toxicities.

OXALIPLATIN AND TAXANES

Oxaliplatin was used in combination with docetaxel in a dose escalation study as first-line treatment of patients with advanced breast cancer [131]. Seven (27%) of 26 patients experienced partial responses. The dose limiting events (at docetaxel in a dose of 75 mg/m² and oxaliplatin in a dose of 80 mg/m²) were neutropenia, diarrhea and fatigue. The treatment was generally well tolerated. Thus, this combination seemed to be active in breast cancer. However, the activity was lower than that of the combinations of cisplatin/carboplatin with taxanes.

PLATINUM COMPLEXES AND ANTI-p185^{HER2/NEU} MONOCLONAL ANTIBODY

The HER2/neu gene encodes a 185-kd receptor tyrosine kinase that is a member of the type I family of growth factor receptors and is homologous to the epidermal growth factor (EGF). Overexpression of this gene is found in approximately 25% of all cancers and is associated with many adverse prognostic factors in breast cancer (see above). In the sera of approximately 20-25% of patients with locally advanced or metastatic breast cancer a soluble form of the extracellular domain (ECD) of p185^{HER2/neu} shed from the tumor cell surface can be detected. Shed HER2/neu ECD positive patients show decreased response to hormonal therapy and have a shortened overall survival compared to shed HER2/neu ECD negative patients. A humanized murine anti-p185^{HER2/neu} monoclonal antibody has shown growth inhibitory activity against HER2/neu overexpressing cell lines and xenografts. The combination of this antibody triggered synergistic effects with cisplatin in HER2/neu overexpressing breast cancer cell lines and tumor xenografts. Furthermore, overexpression of HER2/neu has been associated with resistance to cisplatin.

In a phase II study on 37 patients refractory to chemotherapy treatment with HER2/neu-overexpressing metastatic breast cancer the anti-p185^{HER2/neu} monoclonal antibody (rhMAb HER2, trastuzumab, herceptin) was used together with cisplatin [132]. Patients received a 250 mg loading dose at the first day followed by weekly doses of 100 mg for 9 weeks. Cisplatin at a dose of 75 mg/m² was administered on days 1, 29 and 57. Twenty-four percent partial responses and

24% minor responses or stable diseases were achieved with this regimen. The responding patients showed a significant decrease in serum HER2/neu ECD in contrast to the non-responding patients who showed rising serum levels. Additionally to this promising results no increase in toxicity was observed.

The combination of platinum complexes with trastuzumab also showed high activity in multiple drug combinations. In HER2-overexpressing advanced breast cancer patients docetaxel (75 mg/m²), trastuzumab (4 mg/kg starting dose and then 2 mg/kg for the rest of the treatment) and cisplatin (75 mg/m²) or carboplatin (AUC 6 mg/mL/min) were administered [133]. The treatment was well tolerated and the response rates were high. In the cisplatin group 79% (49 of 62) and in the carboplatin group 58% (34 of 59) overall response rates were achieved.

Similar positive results were reported in a phase II study on the combination of trastuzumab with paclitaxel and carboplatin [134]. In this study patients were initially treated with varying doses of trastuzumab and treated with paclitaxel / carboplatin / trastuzumab or paclitaxel / carboplatin when the disease was stable or progressed. For the paclitaxel / carboplatin / trastuzumab group 84% and the paclitaxel / carboplatin group 69% response rates were reported.

PLATINUM COMPLEXES IN MULTIPLE DRUG COMBINATIONS

Platinum complexes have been investigated in many clinical studies in form of multiple drug combination regimens (besides those already mentioned in the above "trastuzumab" section). For obvious reasons the influence of the platinum compounds on the results of these trials is difficult to evaluate.

SUMMARY

Platinum complexes trigger antiproliferative / cytotoxic effects in human breast cancer cells. IC₅₀-values are found in the low micromolar and nanomolar range. Cellular uptake studies indicate a passive diffusion mechanism for cisplatin in breast cancer cells. The intracellular platinum concentrations depend on the structures of the ligands, the extracellular concentration and the drug exposure period. Less than 10% of the platinum taken up into the cells reaches the cell nuclei.

Synergistic effects in breast cancer cells have been reported for the use of platinum complexes in combination with other agents. Thus, combinations of cisplatin / carboplatin with taxanes or the antibody trastuzumab proved to be more effective than the single agents.

Many efforts have been undertaken to develop platinum complexes based on the drug targeting concept using ligands derived from hormones and anti-hormones. Such agents could be useful in the therapy of estrogen dependent breast cancers. Complexes derived from estrogens showed sufficient cytotoxic effects. The RBA-values for compounds with linkers in the position 3 of the steroid backbone were very low. Complexes with spacers in more suitable positions showed higher RBA-values. Platinum(IV) complexes with estradiol ligands were prepared as prodrugs. Following re-

duction to the respective platinum (II) species inside the cells estradiol is released and sensitizes the cells to the action of the platinum moiety. However, the desired tissue selectivity in proliferation experiments (hormone dependent versus hormone independent breast cancer cells) could not be achieved up to now by estradiol-platinum compounds.

Platinum complexes with triphenylethylene ligands (tamoxifen derivatives) also showed good cytotoxic activities but again without tissue selectivity for hormone dependent breast cancer cells. For some of the compounds antiestrogenic effects in MCF-7 cells were reported.

First reports about platinum complexes exhibiting tissue selective antiproliferative effects were presented by the groups of van Angerer and Schönenberger more than one decade ago. 2-Phenylindole linked platinum drugs were active *in-vitro* and *in-vivo* against hormone dependent but not against hormone independent tissues. Moreover, compounds containing 1,2-diarylethylenediamine ligands exhibited most interesting biological properties. Substances out of this class showed selective antiproliferative effects against hormone dependent tissues *in-vitro* and *in-vivo*. Interestingly, only low RBA-values for these platinum complexes were determined. The *in-vivo* potency of the compounds could be referred to estrogenic properties causing an uterotrophic effect or by a reduction of the endogenous estrogen level.

Phase I or phase II studies on these compounds have not been performed yet. Clinical studies on the use of platinum compounds against breast cancer focussed mainly on cisplatin and carboplatin.

If platinum complexes were used as single agents for a first-line therapy in previously untreated patients high response rates (up to 50% using cisplatin) were observed. These studies are in strong contrast to others describing the use of platinum complexes in pretreated patients. In this case, the response rates were dramatically lower (approximately 10% using cisplatin).

The combination of cisplatin with etoposide showed moderate activity but elevated toxicity. For the combination of carboplatin with etoposide the response rates were higher when used as first-line therapy compared to studies in previously treated patients. Platinum complexes administered together with the vinca-alkaloid vinorelbine were active and well tolerated in anthracycline and taxane pretreated patients. The overall results for the combination of platinum complexes with anthracyclines were disappointing. The activities were limited and the toxicities high.

Platinum complexes together with nucleoside analogues were found to have low to moderate activity.

Regimens containing cisplatin / carboplatin together with taxanes showed a high efficiency. Using the cisplatin combinations overall response rates higher than 80% could be achieved. Furthermore, the treatment was well tolerated and active also in heavily anthracycline pretreated patients. Oxaliplatin also exhibited activity if given together with taxanes. However, the efficiency of this combination seems to be lower than that with cisplatin / carboplatin.

Another very promising approach is the use of platinum complexes together with the antibody trastuzumab in

HER2/neu-overexpressing breast cancer patients. In the first clinical trials high activities and good tolerabilities were achieved. Further research on these combinations is definitely necessary.

Based on the above described results the use of platinum complexes against breast cancer seems to be a reasonable therapeutic choice under critical patient selection and / or in combination with other suitable anticancer drugs.

Hormonally active and breast cancer tissue selective platinum compounds investigated in preclinical studies may bring new options for clinical treatment in the future. More intensive research on these compounds is definitely recommended.

REFERENCES

- [1] Boulikas, T.; Vougiouka, M. *Oncol. Rep.*, **2003**, *10*, 1663.
- [2] Galanski, M.; Jakupec, M.A.; Keppler, B.K. *Curr. Med. Chem.*, **2005**, *12*, 2075.
- [3] Raymond, E.; Chaney, S.G.; Taamma, A.; Cvitkovic, E. *Ann. Oncol.*, **1998**, *9*, 1053.
- [4] Crown, J.P. *Semin. Oncol.*, **2001**, *1*, 28.
- [5] Martin, M. *Clin. Breast Cancer*, **2001**, *2*, 190.
- [6] Decatris, M.P.; Sundar, S.; O'Byrne, K.J. *Cancer Treat. Rev.*, **2004**, *4*, 53.
- [7] Bednarski, P.J. *Biochem. Pharmacol.*, **1992**, *43*, 2609.
- [8] Boubakari; Bracht, K.; Neumann, C.; Grünert, R.; Bednarski, P.J. *Arch. Pharm.*, **2004**, *337*, 668.
- [9] Akamatsu, K.-I.; Saito, H.; Tsunenari, T.; Matsumoto, T.; Morikawa, K.; Koizumi, M.; Mitsui, H.; Koizumi, K. *Anticancer Res.*, **1993**, *13*, 2261.
- [10] Gust, R.; Schnurr, B.; Krauser, R.; Bernhardt, G.; Koch, M.; Schmid, B.; Hummel, E.; Schönenberger, H. *J. Cancer Res. Clin. Oncol.*, **1998**, *124*, 585.
- [11] Ghezzi, A.R.; Aceto, M.; Cassino, C.; Gabano, E.; Osella, D. *J. Inorg. Biochem.*, **2004**, *98*, 73.
- [12] Plum, D.; van Waardenburg, R.C.A.M.; Beijnen, J.H.; Schellens, J.H.M. *Cancer Chemother. Pharmacol.*, **2004**, *54*, 71.
- [13] Lindauer, E.; Holler, E. *Biochem. Pharmacol.*, **1996**, *52*, 7.
- [14] He, Q.; Liang, C.H.; Lippard, S.J. *Proc. Nat. Acad. Sci. USA*, **2000**, *97*, 5768.
- [15] Hafner, F.; Holler, E.; von Angerer, E. *J. Steroid Biochem. Mol. Biol.*, **1996**, *58*, 385.
- [16] Gandolfi, O.; Blum, J. *Inorg. Chim. Acta*, **1984**, *91*, 257.
- [17] Altman, J.; Castrillo, T.; Beck, W.; Bernhardt, G.; Schönenberger, H. *Inorg. Chem.*, **1991**, *30*, 4085.
- [18] Jackson, A.; Davis, J.; Pither, R.J.; Rodger, A.; Hannon, M.J. *Inorg. Chem.*, **2001**, *40*, 3964.
- [19] Cassino, C.; Gabano, E.; Ravera, M.; Cravotto, G.; Palmisano, G.; Vessieres, A.; Jaouen, G.; Mundwiler, M.; Alberto, R.; Osella, D. *Inorg. Chim. Acta*, **2004**, *357*, 2157.
- [20] Gabano, E.; Cassino, C.; Bonetti, S.; Prandi, C.; Colangelo, D.; Ghiglia, A.L.; Osella, D. *Org. Biomol. Chem.*, **2005**, *3*, 3531.
- [21] Descoteaux, C.; Provencher-Mandeville, J.; Mathieu, I.; Perron, V.; Mandal, S.K.; Asselin, E.; Berube, G. *Bioorg. Med. Chem. Lett.*, **2003**, *13*, 3927.
- [22] Perron, V.; Rabouin, D.; Asselin, E.; Parent, S.; Gaudreault, R.C.; Berube, G. *Bioorg. Chem.*, **2005**, *33*, 1.
- [23] Gagnon, V.; St-Germain, M.E.; Descoteaux, C.; Provencher-Mandeville, J.; Parent, S.; Mandal, S.K.; Asselin, E.; Berube, G. *Bioorg. Med. Chem. Lett.*, **2004**, 5919.
- [24] Chesne, C.; Leclercq, G. *Eur. J. Med. Chem.*, **1986**, *4*, 321.
- [25] Georgiadis, M.P.; Haroutounian, S.A. *Inorg. Chim. Acta*, **1987**, *138*, 249.
- [26] Barnes, K.R.; Kutikov, A.; Lippard, S.J. *Chem. Biol.*, **2004**, *11*, 557.
- [27] Berube, G.; Wheeler, P.; Ford, C.H.J.; Gallant, M.; Tsaltas, Z. *Can. J. Chem.*, **1993**, *71*, 1327.
- [28] He, Y.; Groleau, S.; Gaudreault, R.C.; Caron, M.; Therien, H.M.; Berube, G. *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 2217

- [29] Berube, G.; He, Y.; Groleau, S.; Sene, A.; Therien, H.M.; Caron, M. *Inorg. Chim. Acta*, **1997**, *262*, 139.
- [30] Sene, A.; Berube, G.; Gaudreault, R.C. *Drug Des. Disc.*, **1998**, *15*, 277.
- [31] Top, S.; Kaloun, E.B.; Vessieres, A.; Leclercq, G.; Laios, I.; Ourevitch, M.; Deuschel, C.; MCGlinchey, M.J.; Jaouen, G. *ChemBiochem*, **2003**, *4*, 754.
- [32] Knebel, N.; von Angerer, E. *J. Med. Chem.*, **1988**, *31*, 1675.
- [33] Knebel, N.; von Angerer, E. *J. Med. Chem.*, **1991**, *34*, 2145.
- [34] Wappes, B.; Jennerwein, M.; von Angerer, E.; Schönenberger, H.; Engel, J.; Berger, M.; Wrobel, K.H. *J. Med. Chem.*, **1984**, *27*, 1280.
- [35] Gust, R.; Burgemeister, T.; Mannschreck, A.; Schönenberger, H. *J. Med. Chem.*, **1990**, *33*, 2535.
- [36] Gust, R.; Schönenberger, H. *Arch. Pharm.*, **1993**, *326*, 405.
- [37] Gust, R.; Schönenberger, H.; Klement, U.; Range, K.J. *Arch. Pharm.*, **1993**, *326*, 967.
- [38] Karl, J.; Gust, R.; Spruss, T.; Schneider, M.R.; Schönenberger, H.; Engel, J.; Wrobel, K.H.; Lux, F.; Trebert Haerberlin, S. *J. Med. Chem.*, **1988**, *31*, 72.
- [39] Schlemmer, R.; Spruss, T.; Bernhardt, G.; Schönenberger, H. *Arch. Pharm.*, **1999**, *332*, 59.
- [40] Schlemmer, R.; Spruss, T.; Bernhardt, G.; Schönenberger, H. *Arch. Pharm.*, **1999**, *333*, 69.
- [41] Spruss, T.; Schertl, S.; Schneider, M.R.; Gust, R.; Bauer, K.; Schönenberger, H. *J. Cancer Res. Clin. Oncol.*, **1993**, *119*, 707.
- [42] Gust, R.; Faderl, M.; Schönenberger, H. *J. Cancer Res. Clin. Oncol.*, **2000**, *126*, 647.
- [43] Gust, R.; Niebler, K.H.; Schönenberger, H. *J. Med. Chem.*, **1995**, *38*, 2070.
- [44] Vom Orde, H.D.; Reile, H.; Müller, R.; Gust, R.; Bernhardt, G.; Spruss, T.; Schönenberger, H.; Burgemeister, T.; Mannschreck, A. *J. Cancer Res. Clin. Oncol.*, **1990**, *116*, 434.
- [45] Gust, R.; Schönenberger, H. *Eur. J. Med. Chem.*, **1993**, *28*, 117.
- [46] Reile, H.; Bernhardt, G.; Koch, M.; Schönenberger, H.; Hollstein, M.; Lux, F. *Cancer Chemother. Pharmacol.*, **1992**, *30*, 113.
- [47] Gust, R.; Krauser, R.; Schmid, B.; Schönenberger, H. *Inorg. Chim. Act.*, **1996**, *250*, 203.
- [48] Sergejew, T.F.; Hartmann, R.W. *J. Steroid. Biochem. Mol. Biol.*, **1996**, *58*, 243.
- [49] Schertl, S.; Hartmann, R.W.; Batzl-Hartmann, C.; Bernhardt, G.; Spruss, T.; Beckenlehner, K.; Koch, M.; Krauser, R.; Schlemmer, R.; Gust, R.; Schönenberger, H. *Arch. Pharm.*, **2004**, *337*, 335.
- [50] Schertl, S.; Hartmann, R.W.; Batzl-Hartmann, C.; Bernhardt, G.; Spruss, T.; Beckenlehner, K.; Koch, M.; Krauser, R.; Schlemmer, R.; Gust, R.; Schönenberger, H. *Arch. Pharm.*, **2004**, *337*, 349.
- [51] Gust, R.; Niebler, K.; Schönenberger, H. *J. Med. Chem.*, **2005**, *48*, 7132.
- [52] Martin, M.B.; Reiter, R.; Pham, T.; Avellanet, Y.R.; Camara, J.; Lahm, M.; Pentecost, E.; Pratap, K.; Gilmore, B.A.; Divekar, S.; Dagata, R.S.; Bull, J.L.; Stoica, A. *Endocrinology*, **2003**, *144*, 2425.
- [53] Stoica, A.; Katzenellenbogen, B.S.; Martin, M.B. *Mol. Endocrinol.*, **2000**, *14*, 545.
- [54] Johnson, M.D.; Kenney, N.; Stoica, A.; Hilakivi-Clarke, L.; Singh, B.; Chepko, G.; Clarke, R.; Sholler, P.F.; A Lirio, A.; Foss, C.; Reiter, R.; Trock, B.; Paik, S.; Martin, M.B. *Nature Med.*, **2003**, *9*, 1081.
- [55] Leistner, E. *Pharm. Unserer Zeit*, **2005**, *34*, 98.
- [56] Bartsch, V. *Pharm. Unserer Zeit*, **2005**, *34*, 104.
- [57] Kodali, S.; Burkley, M.; Nag, K.; Taylor, R.C.; Moudgil, V.K. *Biochem. Biophys. Res. Commun.*, **1994**, *202*, 1413.
- [58] Bogdanovic, G.; Kojic, V.; Srdic, T.; Jakimov, D.; Djuran, M.I.; Bugarcic, Z.D.; Baltic, M.; Baltic, V. *Metal-based Drugs*, **2002**, *9*, 33.
- [59] Frasci, G.; Comella, P.; D'Aiuto, G.; Budillon, A.; Barbarulo, D.; Thomas, R.; Capasso, I.; Casaretti, R.; Daponte, A.; Caponigro, F.; Gravina, A.; Maiorino, L.; Carateni, G.; Gentile, A.; Omella, G. *Breast Cancer Res. Treat.*, **1998**, *49*, 13.
- [60] Konecny, G.; Untch, M.; Slamon, D.; Beryt, M.; Kahlert, S.; Felber, M.; Langer, E.; Lude, S.; Hepp, H.; Pegram, M. *Breast Cancer Res. Treat.*, **2001**, *67*, 223.
- [61] Slamon, D.J.; Clark, G.M.; Wong, S.J.; Levin, W.J.; Ullrich, A.; McGuire, W.L. *Science* **1987**, *235*, 177.
- [62] Slamon, D.; Pegram, M. *Semin. Oncol.*, **2001**, *28*, 13.
- [63] Pietras, R.J.; Fendly, B.M.; Chazin, V.R.; Pegram, M.D.; Howell, S.B.; Slamon, D.J. *Oncogene* **1994**, *9*, 1829.
- [64] Pegram, M.; Hsu, S.; Lewis, G.; Pietras, R.; Beryt, M.; Sliwkowski, M.; Coombs, D.; Baly, D.; Kabbavar, F.; Slamon, D. *Oncogene* **1999**, *18*, 2241.
- [65] Arteaga, C.L.; Winnier, A.R.; Poirier, M.C.; Lopez-Larraz, D.M.; Shawver, L.K.; Hurd, S.D.; Stewart, S.J. *Cancer Res.*, **1994**, *54*, 3758.
- [66] Pietras, R.J.; Pegram, M.D.; Finn, R.S.; Manrval, D.A.; Slamon, D.J. *Oncogene*, **1998**, *17*, 2235.
- [67] Pegram, M.D.; Konecny, G.E.; O'Callaghan, C.; Beryt, M.; Pietras, R.; Slamon, D.J. *J. Natl. Cancer Inst.*, **2004**, *739*.
- [68] Konecny, G.E.; Pegram, M.D. *Oncology*, **2004**, *18*(14, Suppl), 32.
- [69] Adjei, A.A.; Davis, J.N.; Bruzek, L.M.; Ehrlichmann, C.; Kaufmann, S.H. *Clin. Cancer Res.* **2001**, *7*, 1438.
- [70] Raymond, E.; Buquet-Fagot, C.; Djelloul, S.; Mester, J.; Cvitkovic, E.; Allain, P.; Louvet, C.; Gaspach, C. *Anticancer Drugs*, **1997**, *8*, 876.
- [71] Otto, A.M. *J. Cancer Res. Clin. Oncol.*, **1994**, *120*, 286.
- [72] Saito, T.; Berens, M.E.; Welander, C.E. *Cancer Chemother. Pharmacol.*, **1987**, *19*, 233.
- [73] Schumacher, K. *Therapie maligner Tumoren*, Schattauer-Verlag: Stuttgart, **2000**.
- [74] Kolaric, K.; Roth, A. *Cancer Chemother. Pharmacol.*, **1983**, *13*, 142.
- [75] Sledge G.W.; Loehrer P.L.; Roth B.J.; Einhorn, L.H. *J. Clin. Oncol.*, **1988**, *6*, 1811.
- [76] Martino, S.; Samal, B.A.; Singhakowinta, A.; Yoshida, S.; Mackenzie, M.; Jain, J.; Vaitkevicius, V.K. *J. Cancer Res. Clin. Oncol.*, **1984**, *108*, 354.
- [77] Yap, H.Y.; Salem, P.; Hortobagyi, G.N.; Bodey, G.P.; Budzar, A.U.; Tashima, C.K.; Blumenschein, G.R. *Cancer Treat. Rep.*, **1978**, *62*, 405.
- [78] Vermorken, J.B.; Gundersen, S.; Clavel, M.; Smyth, J.F.; Dodion, P.; Renard, J.; Kaye, S.B. *Ann. Oncol.*, **1993**, *4*, 303.
- [79] Kolaric, K.; Vukas, D. *Cancer Chemother. Pharmacol.*, **1991**, *27*, 409.
- [80] Martin, M.; Diaz-Rubio, E.; Casado, A.; Santabarbara, P.; Lopez Vega, M.; Adrover, E.; Lenaz, L. *J. Clin. Oncol.*, **1992**, *10*, 433.
- [81] O'Brien, M.E.R.; Talbot, D.C.; Smith, I.E. *J. Clin. Oncol.*, **1993**, *11*, 2112.
- [82] Mathe, G.; Kidani, Y.; Triana, K.; Brienza, S.; Ribaud P.; Goldschmidt, E.; Ecstein E.; Despax, R.; Musset, M.; Misset, J.L. *Bio-med. Pharmacother.*, **1986**, *40*, 372.
- [83] Caussanel, J.P.; Levi, F.; Brienza, S.; Misset, J.L.; Itzhaki, M.; Adam, R.; Milano, G.; Hecquet, B.; Mathe, G. *J. Natl. Cancer Inst.*, **1990**, *82*, 1046.
- [84] Garufi, C.; Nistico, C.; Brienza, S.; Vaccaro, A.; D'Ottavio, A.; Zappala, A.R.; Aschelter, A.M.; Terzoli, E. *Ann. Oncol.*, **2001**, *12*, 179.
- [85] Casper, E.S.; Smart, T.C.; Hakes, T.B.; Ochoa Jr., M.; Kaufmann, R. *J. Invest. New Drugs*, **1988**, *6*, 87.
- [86] Meisner, D.J.; Ginsberg, S.; Ditch, A.; Louie, A.; Newman, N.; Comis, R.; Poesz, B. *Am. J. Clin. Oncol.*, **1989**, *12*, 129.
- [87] Theriault R.L.; Walters R.S.; Holmes, F.A.; Esparza-Guerra, L.; Kowal, C.; Hortobagyi G.N. *Cancer Chemother. Pharmacol.*, **1996**, *38*, 289.
- [88] Fukoka, M.; Niitani, H.; Hasegawa, K.; Majima, J.; Hino, M.; Furue, H.; Tsukagoshi, S.; Fujita, H.; Ohta, K.; Furuse, K.; Kimura, S.; Katoh, T. *Proc. Am. Soc. Clin. Oncol.*, **1989**, *8*, 62.
- [89] Kraker, A.; Moore, C.; Leopold, W.; Takahashi, K. *Proc. Am. Assoc. Cancer Res.*, **1988**, *344*, 1370.
- [90] Theriault, R.L.; Cohen, I.A.; Esparza, L.; Kowal, C.; Raber, M.N. *Cancer Chemother. Pharmacol.*, **1992**, *31*, 333.
- [91] Gelmon, K.A.; Vandenberg, T.A.; Panasci, L.; Norris, B.; Crump, M.; Douglas, L.; Walsh, W.; Matthews, S.J.; Seymour, L.K. *Ann. Oncol.*, **2003**, *14*, 543.
- [92] Kurata, T.; Tamura, T.; Sasaki, Y.; Fujii, H.; Negoro, S.; Fukuoka, M.; Saijo, N. *Jpn. J. Clin. Oncol.*, **2000**, *30*, 377.
- [93] Aoyama H.; Kubo, K.; Uchino, J.; Hayasaka, H.; Asaishi, K.; Izuo, M.; Ogawa, M.; Majima, H.; Yasutomi, M.; Wada, T.; Monden, Y.; Tamura, K.; Isogai, Y.; Watanabe, H.; Yoshida, M.; Miyazaki, I.; Nagasue, N.; Takashima, S.; Ota, K. *Jpn. J. Cancer Chemother.*, **1992**, *19*, 1033.
- [94] Koyama, H.; Ogawa, M.; Kuraishi, Y.; Tominaga, K.; Yoshida, M.; Taguchi, T. *Jpn. J. Cancer Chemother.*, **1992**, *19*, 1049.

- [95] Tanis, B.C.; Vermorken, J.B.; Huinink, W.W.T.B.; Klein, I.; Gall, H.; Van Oosterom, A.T.; Simonetti, G.; Mc Vie, J.G.; Van der Vijgh, W.J.F.; Pinedo, H.M. *Eur. J. Cancer*, **1991**, *27*, 268.
- [96] Schabel, F.M.; Trader, M.W.; Laster, W.R.; Corbett, T.H.; Griswold, D.P. *Cancer Treat. Rep.*, **1979**, *63*, 1459.
- [97] Durand, R.E.; Goldie, J.H. *Cancer Treat. Rep.*, **1987**, *71*, 673.
- [98] Krook, J.E.; Loprinzi, C.L.; Schaid, D.J.; Kardinal, C.G.; Maillard, J.A.; Pfeifle, D.M.; Ellison, N.M.; Reuter, N.F.; Nelimark, R.A. *Cancer*, **1990**, *65*, 418.
- [99] Ceci, G.; Bisagni, G.; Cocconi, G.; Rodino, C.; Belsanti, V.; Bertusi, M.; Buzzi, F.; Bacchi, M. *Tumori*, **1995**, *81*, 241.
- [100] Cocconi, G.; Bisagni, G.; Bacchi, M.; Boni, C.; Bartolucci, R.; Ceci, G.; Colozza, M.A.; De Lisi, V.; Lottici, R.; Mosconi, A.M.; Passalacqua, R.; Tonato, M. *J. Clin. Oncol.*, **1991**, *9*, 664.
- [101] Lluch, A.; Azagra, P.; Cervantes, A.; Munoz, M.; Alberola, V.; Santabarbara, P.; Garcia-Conde, J. *Oncology*, **1994**, *51*, 352.
- [102] Crown, J.; Hakes, T.; Reichman, B.; Lebwahl, D.; Gilewski, T.; Surbone, A.; Currie, V.; Yao, T.J.; Hudis, C.; Seidman, A.; Norton, L. *Cancer*, **1993**, *71*, 1254.
- [103] Van der Gaast, A.; Bontenbal, M.; Planting, A.S.; Kok, T.C.; Splinter, T.A. *Ann. Oncol.*, **1994**, *5*, 858.
- [104] Vinolas, N.; Daniels, M.; Estate, J.; Grau, J.J.; Palombo, H.; Sola, C. *Am. J. Clin. Oncol.*, **1992**, *15*, 160.
- [105] Ray-Coquard, I.; Biron, P.; Bachelot, T.; Guastalla, J.P.; Catimel, G.; Merrouche, Y.; Droz, J.P.; Chauvin, F.; Blay, J.Y. *Cancer*, **1998**, *82*, 134.
- [106] Shamseddine, A.; Taher, A.; Dabaja, B.; Azzam, D.; Ziad, S.; El Saghier, N. *Am. J. Clin. Oncol.*, **1999**, *22*, 298.
- [107] Kosmas, C.; Agelaki, S.; Giannakakis, T.; Mavroudis, D.; Kouroussis, C.; Kalbakis, K.; Papadouris, S.; Souglakos, J.; Malamos, N.; Georgoulas, V. *Oncology*, **2002**, *62*, 103.
- [108] Iaffaioli, R.V.; Tortoriello, A.; Facchini, G.; Santangelo, M.; De Sena, G.; Gesue, G.; Bucci, L.; Scaramellino, G.; Anastasio, E.; Finizio, A.; *et al. Br. J. Cancer*, **1995**, *72*, 1256.
- [109] Kakolyris, S.; Kouroussis, C.; Koukourakis, M.; Mavroudis, D.; Malas, K.; Vardakis, N.; Bozionelou, V.; Kalbakis, K.; Georgoulas, V. *Oncology*, **2002**, *63*, 213.
- [110] Atiba, J.O.; Green, S.J.; Hynes, H.E.; Osborne, C.K.; Miller, T.P.; Davidner, M. *Invest. New Drugs*, **1994**, *12*, 129.
- [111] Thurlimann, B.; Senn, H.J.; Jungi, W.F. *J. Cancer Res. Clin. Oncol.*, **1990**, *116*, 13.
- [112] Casper, E.S.; Curley, T.; Hakes, T.B. *Invest. New Drugs*, **1989**, *7*, 189.
- [113] Oster, M.W.; Schilsky, R.L.; Faraggi, D.; Korzun, A.H.; Perry, M.; Moore, A.; Kalra, J.M.; Wood, W.C.; Henderson, I.C. *Cancer*, **1991**, *68*, 1696.
- [114] Nagourney, R.A.; Link, J.S.; Blitzer, J.B.; Forsthoft, C.; Evans, S.S. *J. Clin. Oncol.*, **2000**, *18*, 2245.
- [115] Spaeth, D.; Conroy, T.; Krakowski, I.; Geoffrois, L.; Luporsi, E.; Rios, M.; Weber, B. *Bull. Cancer*, **1993**, *80*, 351.
- [116] Palacio, I.; Buesa, J.M.; De Sande, L.; Cueva, J.F.; Esteban, E.; Estrada, E.; Gracia, J.M.; Lacave A.J. *Eur. J. Cancer*, **1992**, *28*, 242.
- [117] Zelek, L.; Cottu, P.; Tubiana-Hulin, M.; Vannetzel, J.-M.; Chollet, P.; Misset, J.-L.; Chouaki, N.; Marty, M.; Gamelin, E.; Culine, S.; Dieras, V.; Mackenenzie, S.; Spielman, M. *J. Clin. Oncol.*, **2002**, *20*, 2551.
- [118] Thuss-Patience, P.C.; von Minckwitz, G.; Kretschmar, A.; Loibl, S.; Schaller, G.; Dorken, B.; Reichardt, P. *Anticancer-Drugs*, **2003**, *14*, 549.
- [119] Gourley, C.; Cassidy, J.; Edwards, C.; Samuel, L.; Bisset, D.; Camboni, G.; Young, A.; Boyle, D.; Jodrell, D. *Cancer Chemother. Pharmacol.*, **2004**, *53*, 95.
- [120] Schiff, P.B.; Fant, J. *Nature*, **1979**, *277*, 665.
- [121] Schiff, P.B.; Horwitz, S.B. *Proc. Natl. Acad. Sci. USA*, **1980**, *77*, 1561.
- [122] Crown, J.; Pegram, M. *Breast Cancer Res. Treat.*, **2003**, *79* (Suppl. 1), S11.
- [123] Park, S.H.; Cho, E.K.; Bang, S.M.; Shin, D.B.; Lee, J.H.; Lee, Y.D. *BMC Cancer*, **2005**, *5*, 21.
- [124] Gelmon, K.A.; O'Reilly, S.E.; Tolcher, A.W.; Campbell, C.; Bryce, C.; Ragaz, J.; Coppin, C.; Plenderleith, I.H.; Ayers, D.; McDermott, B.; Nakashima, L.; Healey, D.; Onetto, N. *J. Clin. Oncol.*, **1996**, *14*, 1185.
- [125] Sparano, J.A.; Neuberg, D.; Glick, J.H.; Robert, N.J.; Goldstein, L.J.; Sledge, G.W.; Wood, W. *J. Clin. Oncol.*, **1997**, *15*, 1880.
- [126] Wasserheit, C.; Frazzin, A.; Oratz, R.; Sorich, J.; Downey, A.; Hochster, H.; Chachoua, A.; Wernz, J.; Zeleniuch-Jacquotte, A.; Blum, R.; Speyer, J. *J. Clin. Oncol.*, **1996**, *14*, 1993.
- [127] Fountzilas, G.; Dimopoulos, A.M.; Papadimitriou, C.; Kalogera-Fountzila, A.; Aravintos, G.; Bafaloukos, D.; Athanassiades, A.; Nicolaidis, C.; Keramopoulos, A.; Pavlidis, N.; Kosmidis, P.; Skarlos, D. *Ann. Oncol.*, **1998**, *9*, 1031.
- [128] Perez, E.A.; Hillman D.W.; Stella, P.J.; Krook, J.E.; Hartmann, L.C.; Fitch, T.R.; Hatfield, A.K.; Mailliard, J.A.; Nair, S.; Kardinal, C.G.; Ingle J.N. *Cancer*, **2000**, *88*, 124.
- [129] Fountzilas, G.; Athanassiades, A.; Kalogera-Fountzila, A.; Aravantinos, G.; Bafaloukos, D.; Briasoulis, E.; Dombros, N.; Ioannidis, I.; Pavlidis, N.; Kosmidis, P.; Skarlos, D. *Eur. J. Cancer*, **1997**, *33*, 1893.
- [130] Loesch, D.; Robert, N.; Asmar, L.; Gregurich, M.A.; O'Rourke, M.; Dakhil, S.; Cox, E. *J. Clin. Oncol.*, **2002**, *20*, 3857.
- [131] Kouroussis, C.; Agelaki, S.; Mavroudis, D.; Kakolyris, S.; Androulakis, N.; Kalbakis, K.; Souglakos, J.; Mallas, K.; Bozionelou, V.; Pallis, A.; Adamtziki, H.; Georgoulas, V. *Anticancer Res.*, **2003**, *23*, 785.
- [132] Pegram, M.D.; Lipton, A.; Hayes, D.F.; Weber, B.L.; Baselga, J.M.; Tripathy, D.; Baly, D.; Baughman, S.A.; Twaddell, T.; Glaspy, J.A.; Slamon, D.J. *J. Clin. Oncol.*, **1998**, *16*, 2659.
- [133] Pegram, M.D.; Pienkowski, T.; Northfelt, D.W.; Eiermann, W.; Patel, R.; Fumoleau, P.; Quan, E.; Crown, J.; Toppmeyer, D.; Smylie, M.; Riva, A.; Blitz, S.; Press, M.F.; Reese, D.; Lindsay, M.-A.; Slamon, D.J. *J. Nat. Cancer Inst.*, **2004**, *96*, 759.
- [134] Burris, H.; Yardley, D.; Jones, S.; Houston, G.; Broome, C.; Thompson, D.; Greco, F.A.; White, M.; Hainsworth, J. *J. Clin. Oncol.*, **2004**, *22*, 1621.