Tamoxifen Derivatives for Delivery of the Antitumoral (DACH)Pt Group: Selective Synthesis by McMurry Coupling, and Biochemical Behaviour**

Siden Top,*[a] El Bachir Kaloun,[a] Anne Vessières,[a] Guy Leclercq,[b] Ioanna Laïos,[b] Michèle Ourevitch,[c] Christine Deuschel,[d] Michael J. McGlinchey,[e] and Gérard Jaouen*[a]

*The goal of our study was to potentiate the effects of the ((*R,R)-trans-*1,2-diaminocyclohexane)-platinum(II) fragment [(DACH)Pt], known for its cytotoxic properties, either with tamoxifen (Tam), the most widely used antiestrogen in the treatment of hormone-dependent breast cancers, or with its active metabolite hydroxytamoxifen (hydroxy-Tam). We coupled Tam or hydroxy-Tam derivatives bearing a malonato group at the* para *position of the* β *aromatic ring with the (DACH)Pt fragment. The malonato-Tam and malonato-hydroxy-Tam compounds were prepared through McMurry coupling of the appropriate ketones. The presence of the malonate group resulted in a pronounced stereospecificity in the reaction, since malonato-Tam was obtained only as the* Z *isomer, while malonato-hydroxy-Tam was obtained as an 80/20* E/Z *mixture. Attribution of the isomeric structures was achieved by 2D NMR spectroscopy. The platinum complexes (DACH)Pt-malonato-Tam and (DACH)Pt-malonato-hydroxy-Tam were then prepared by coupling the barium salts derived from the malonato-Tam and malonato-hydroxy-Tam with the nitrate derived from (DACH)PtCl$_2$. Study of the biochemical properties of these two platinum complexes showed that, while the hydroxy-Tam complex is satisfactorily recognized by the estrogen receptor (relative binding affinity, RBA = 6.4 %), the Tam complex is less well recognized (RBA = 0.5 %). The effects of these complexes on two hormone-dependent breast cancer cell lines (MCF7 and MVLN) were studied in vitro. Both complexes showed an antiproliferative effect on MCF7 cells, and an antiestrogenic effect on MVLN cells. The observed effects appear to be essentially antihormonal, since incorporation of the (DACH)Pt fragment into the tamoxifen skeleton did not cause an increase in the cytotoxicity of the complexes.*

KEYWORDS:

antitumor agents · bioinorganic chemistry · breast cancer · platinum · tamoxifen

Introduction

Chemotherapeutics often have to deal with the nonspecificity of available drugs, which may affect healthy as well as tumorous cells and give rise to secondary effects of varying severity. To overcome this problem, the active principle may be targeted to a specific site by use of, for example, hormone – receptor or high-affinity antigen – antibody systems. Estradiol, bound to its specific receptor, plays an important role in the development of certain hormone-dependent cancers, which include two out of three breast cancers.[1–3] Tamoxifen, the archetypal selective estrogen receptor modulator (SERM), is the drug most commonly used to combat these cancers. Its active metabolite, hydroxytamoxifen, binds to the estrogen receptor with a relative binding affinity almost 100 times higher than that of tamoxifen and produces enhanced antiestrogenic activity in vitro.[4–8] We have recently shown that ferrocene, an organometallic compound, can be used to potentiate the activity of tamoxifen when a ferrocenyl moiety is substituted for the aromatic β ring of tamoxifen to yield the ferrocifens.[9, 10]

The hydroxyferrocifens show an activity at least equal to that of tamoxifen in the case of cell lines containing the estrogen α

[a] *Dr. S. Top, Prof. G. Jaouen, Dr. El B. Kaloun, Dr. A. Vessières*
Laboratoire de Chimie Organométallique
UMR CNRS 7576
Ecole Nationale Supérieure de Chimie de Paris
11 rue Pierre et Marie Curie, 75231 Paris 05 (France)
Fax (+ 33) 1-43-26-00-61
E-mail: gerard-jaouen@enscp.jussieu.fr

[b] *Prof. G. Leclercq, I. Laïos*
Laboratoire de Cancérologie Mammaire
Institut Jules Bordet, Rue Héger Bordet
Bruxelles, 1000 (Belgium)

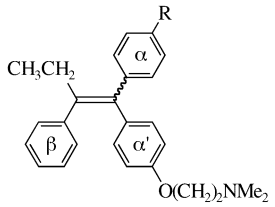
[c] *Dr. M. Ourevitch*
Laboratoire de Chimie Thérapeutique BIOCIS
UPRESA CNRS 8076
Faculté des Sciences Pharmaceutiques et Biologiques
Université Paris XI, 5 rue Jean-Baptiste Clément
92290 Chatenay – Malabry (France)

[d] *Dr. C. Deuschel*
Debiopharm S.A., rue des Terreaux 17
Case postale 211, 1000 Lausanne 9 (Switzerland)

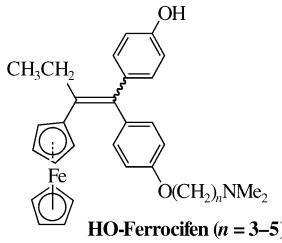
[e] *Prof. M. J. McGlinchey*
Department of Chemistry, University College Dublin
Belfield, Dublin 4 (Ireland)

[**] *(DACH)Pt, ((R,R)-trans-*1,2-diaminocyclohexane)-platinum(II).

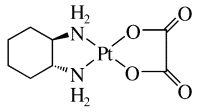
 © 2003 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim DOI: 10.1002/cbic.200200550



Tamoxifen (R = H)
HO-Tamoxifen (R = OH)



HO-Ferrocifen (n = 3–5)



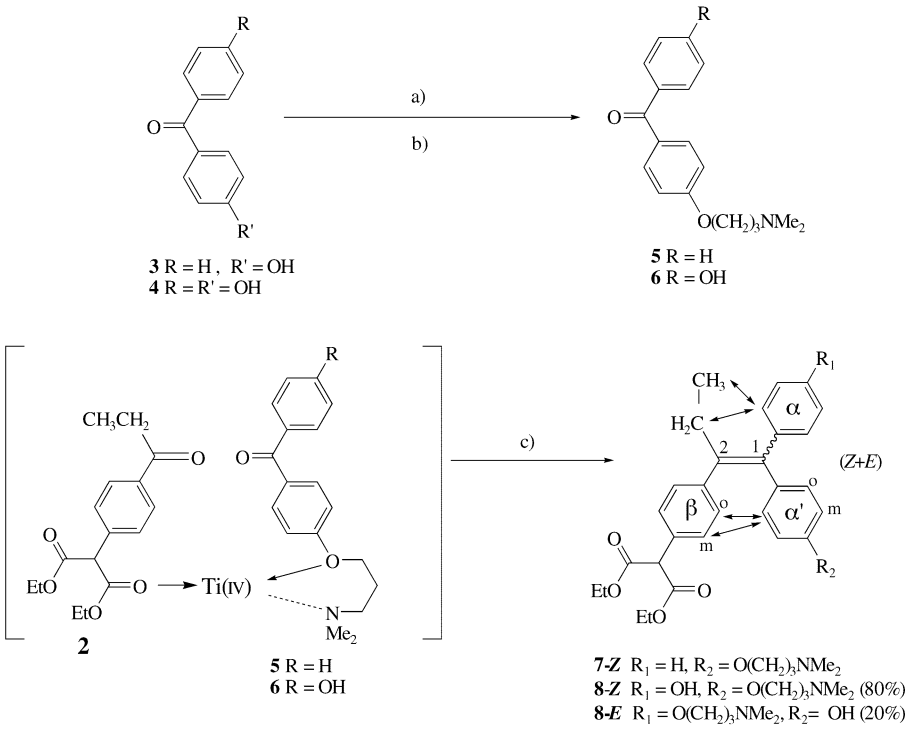
Oxaliplatin

receptor (for example, the MCF7 cell line), but their most interesting characteristic is their antiproliferative activity on cell lines that do not contain the α form of the estrogen receptor, but instead contain the β form (such as MDA-MB231[11]). This discovery provided the impetus to examine the behaviour of other transition metals in the same context.[12, 13] Of the transition metals, platinum is the most widely used in oncology. It is administered in the form of cisplatin [*cis*-dichlorodiammineplatinum(II)], a drug discovered by Rosenberg more than thirty years ago,[14–16] and is particularly efficacious against testicular and ovarian cancers. We therefore felt it would be interesting to synthesize platinum complexes attached to the tamoxifen framework. Care is required, however, in selecting the ligands to be bonded to platinum when designing a therapeutic molecule. Cisplatin itself is not without disadvantages, notably various secondary effects that are not well tolerated by patients, together with its lack of effectiveness against a number of cancers, notably breast cancer. In addition, attempts to couple cisplatin to derivatives of estradiol or nonsteroidal estrogen did not give conclusive results.[17–19] Another consideration is that of resistance, which may build up after prolonged use of the drug. To overcome these problems, researchers have synthesized a very large number of analogues of cisplatin.[20–23] Of the thousands of new molecules synthesized, only about 30 have succeeded in reaching the clinical trial stage,[5, 24] and of these only half a dozen have received approval for clinical use. Two are currently in routine use: carboplatin [*cis*-diammine-1,1′-cyclobutanedicarboxylate platinum(II)] and oxaliplatin [(*R*,*R*)-*trans*-1,2-diaminocyclohexaneoxaloplatinum(II)].

Carboplatin is a distinct improvement over cisplatin, showing similar activity but with lower toxicity. Oxaliplatin, in which the ((*R*,*R*)-*trans*-1,2-diaminocyclohexane)platinum fragment—(DACH)Pt—is bonded to an oxalate ligand, is available under the tradenames Eloxatine, Dacplat, or Dacotin and is a third generation drug effective against certain cisplatin-resistant cancers such as colorectal cancer.[25–29] Biochemical tests have shown moderate antiproliferative activity for oxaliplatin on MCF7 cells derived from a hormone-dependent breast cancer with an IC_{50} value (concentration required to give 50% inhibition) of 7.4 μM.[30] We therefore decided to test the viability of using tamoxifen, a known effective antiestrogen, as the vector for delivery of a recognized cytotoxic fragment, (DACH)Pt, into hormone-dependent breast cancer cells. Here we report the synthesis and 2D NMR spectroscopy identification of derivatives of tamoxifen or hydroxytamoxifen substituted at the *para* position of the β aromatic ring by a malonato moiety that is in turn complexed to the (DACH)Pt fragment. This synthesis was followed by a study of biological properties of these compounds, specifically their affinity for the estradiol receptor and their in vitro effects on hormone-dependent breast-cancer-derived cell lines (MCF7 and MVLN).

Results and Discussion

The first step of the synthetic sequence is the preparation by McMurry coupling of the malonates **7** and **8** (Scheme 1), derived from tamoxifen and hydroxytamoxifen, respectively, followed by ester hydrolysis and formation of the corresponding barium salts **11** and **12** (Scheme 2, see below). Malonate is used here in place



Scheme 1. *Synthetic route to complexes **7** and **8**; the double-headed arrows indicate observed NOE interactions between the indicated protons. a) NaH, tetrahydrofuran (THF); b) dimethylformamide (DMF), $Cl(CH_2)_3NMe_2HCl$; c) $TiCl_4$, Zn, THF.*

 © 2003 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

of the oxalate ligand of oxaliplatin because an extra carbon atom is required in order to provide a link to the tamoxifen skeleton. The nitrate salt (DACH)Pt(NO_3)$_2$ (**10**), prepared concurrently, is then coupled to the malonates **11** and **12** to give the expected products (DACH)Pt-malonato-Tam (**13**) and (DACH)Pt-malonato-hydroxy-Tam (**14**), respectively.

Synthesis of the tamoxifen- and hydroxytamoxifen-derived malonates 7 and 8

The McMurry coupling procedure has proved very effective in the synthesis of ethylenic compounds such as tamoxifen.[31] This reaction has also been successfully applied in our laboratory to the syntheses of ferrocifens, the ferrocenyl analogues of tamoxifen.[10, 32] Normally a mixture of *Z* and *E* isomers is obtained, with only moderate selectivity. Owing to its relative ease of employment, we also selected this synthetic strategy for the synthesis of the desired platinum compounds.

Diethyl (4-propionylphenyl)malonate (**2**), prepared by a Friedel – Crafts reaction between ethyl phenylmalonate (**1**) and propionyl chloride in the presence of $AlCl_3$ in dichloromethane at 60 °C, furnished only the *para*-substituted compound in 95% yield (Scheme 1). The ketones 4-hydroxybenzophenone (**3**) and 4,4′-dihydroxybenzophenone (**4**) were functionalized to give **5** and **6**, respectively, by treatment with 3-chloropropyldimethylammonium chloride. The reaction with 4,4′-dihydroxybenzophenone has previously been reported to give a mixture of mono- and disubstituted products,[9] but this problem was avoided in the current case by carrying out the reaction in DMF and by use of NaH as the base, which gave essentially only the monosubstituted ketones **5** and **6**. Finally, the McMurry coupling reaction between the malonic derivative **2** and ketone **5** or **6** in the presence of the reagent $TiCl_4$/Zn in THF gives **7** and **8**. In the case of ketone **5** the reaction is very selective, giving almost exclusively the isomer **7-*Z***, structurally determined by NMR spectroscopy (see below). With ketone **6**, however, the coupling is less selective and an 80/20 *E*/*Z* mixture is obtained.

Determination of the structure of 7 by NMR spectroscopy

Gauthier et al. recently succeeded in identifying the *Z* and *E* isomers of hydroxytamoxifen and toremifen by NMR spectroscopy,[33] specifically from an NOE study that revealed the proximity of the methylene protons of the ethyl group and the *ortho* protons of the neighbouring aromatic rings. Since use of this technique requires definitive attribution of all the aromatic protons, we decided to study compound **7**, the precursor of **13**, since it is a somewhat simpler molecule. Use of other 2D NMR techniques (COSY, NOESY, HMQC, and HMBC) permitted definitive assignment of all the protons and carbon atoms, as well as of the *Z* configuration of the compound.

The NOESY spectrum of **7** shows a nuclear Overhauser interaction between the methine proton of the $CH(CO_2Et)_2$ group, which resonates at 4.52 ppm, and the aromatic proton resonance at 7.20 ppm, which must be the *meta* protons of the β ring (Scheme 1). $^1H-^1H$ COSY together with HMBC and HMQC experiments allow the complete attribution of all the remaining protons and carbon atoms of this aryl ring. Similarly, an NOE correlation between the OCH_2 protons of the amine chain, at $\delta =$ 3.85, and the *meta* protons of the α′ ring (at $\delta = 6.52$) allows one to identify all the other resonances attributable to that particular aryl group. In a similar fashion, all proton and carbon resonances in the molecule were assigned unambiguously. The *Z* configuration attributed to **7** is unequivocally confirmed by the existence of NOE effects not only between the *ortho* protons of the α′ ring and the *ortho* and *meta* protons of the β ring, but also between the *ortho* protons of the α ring and the methylene and methyl groups of the ethyl substituent, as indicated in Scheme 1.

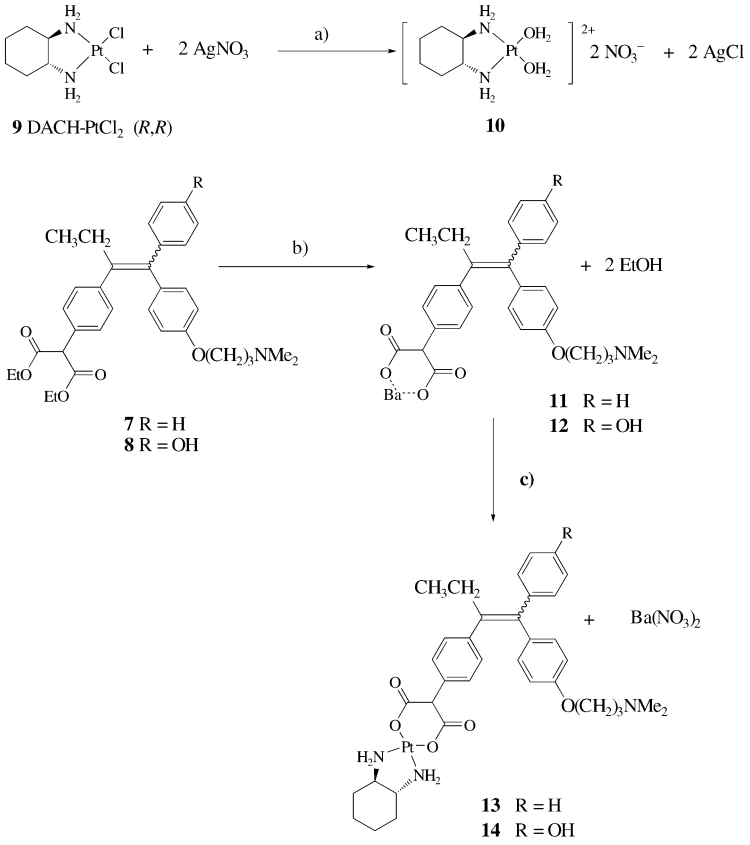
The high stereoselectivity of the coupling reaction between ketones **2** and **5**, with exclusive formation of the isomer **7-*Z***, is perhaps surprising. In contrast, the coupling between **2** and **6** gives a mixture of the two isomers in an 80/20 ratio, with an excess of the isomer **8-*E*** over **8-*Z***, based on the NMR study. The OCH_2 signal of the amine chain is generally observed at a higher field for the *Z* than for the *E* isomer.[32] In fact, the NMR spectrum of the mixture **8** shows an intense triplet at $\delta = 4.04$ and a weak triplet at $\delta = 3.88$ for the OCH_2 protons, suggesting that the **8-*E*** isomer is predominant, a result in complete agreement with that obtained by Gauthier et al.[33] These authors showed that the coupling between propiophenone and dihydroxybenzophenone monoprotected by a trimethylacetoxy group gave predominantly one isomer in an *E*/*Z* ratio of 14:1.

One is tempted to explain these observations in terms of a pre-complexation (see Scheme 1) such that an ester carbonyl group in **2** and the ether oxygen atom (and possibly even the nitrogen atom) in **5** are linked to the same Lewis acid site (in either a titanium or zinc chloride), which would favor the *Z* isomer. In contrast, when the diaryl ketone has two possible donor sites, OH or OR, as in **6**, it is the hydroxy group that takes precedence and determines the more favored stereochemistry.

Synthesis of platinum complexes 13 and 14

The synthetic route used for the preparation of the platinum compounds **13** and **14** is shown in Scheme 2. (DACH)$PtCl_2$ (**9**) was first allowed to react with silver nitrate in water in the dark to give the nitrate salt **10**.[34, 35] One method used for the coupling reaction between the malonate and the nitrate of Pt is to transform the malonate into its barium salt before allowing it to react with the nitrate.[36–38] The malonate derivative **7** was therefore first allowed to react with $Ba(OH)_2 \cdot H_2O$ in aqueous solution, whereupon the barium salt **11** was recovered in the form of a white precipitate, and subsequently allowed to react with the nitrate **10** in water. After the reaction had been allowed to continue overnight, the (DACH)Pt derivative of tamoxifen, **13**, was obtained in 60% yield. This compound is insoluble in organic solvents such as diethyl ether, tetrahydrofuran, or acetone, but is soluble in methanol. The IR spectrum shows the two characteristic peaks of the Pt–OCOR bond at 1630 and 1604 cm^{-1}. The (DACH)Pt derivative of hydroxytamoxifen, **14**, was obtained by the same method from its malonate precursor **8** in 58% yield.

 © 2003 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



Scheme 2. *Synthetic route to complexes **13** and **14**. a) H_2O; b) $Ba(OH)_2$, 8 H_2O, MeOH; c)**10**, H_2O.*

Biochemical studies of the platinum complexes 13 and 14

Biochemical testing was carried out with **13** (the *Z* isomer exclusively) and **14** (mixture of the *Z* and *E* isomers in 20/80 ratio). It is known that the antiproliferative effects of certain platinum-derived drugs, such as cisplatin, are not evident until after hydrolysis, which occurs over several hours.[39] In the case of a malonate ligand bound to platinum, as in complexes **13** and **14**, the hydrolysis is slower; a half-life of 11 days has been reported for a complex of this type (namely, D,L-*trans*-1,2-diaminocyclohexane)malonatoplatinum(II).[40]

Measurement of relative binding affinity (RBA) for the estradiol receptor

The first biochemical test performed was to determine whether or not the new compounds retained their binding affinity for the estradiol alpha receptor, by measurement of their RBA values for this receptor. As would be expected, compound **14**, which bears an OH group, is adequately recognized by the estradiol receptor (RBA = 6.4). This value, however, is lower than that found for the malonate precursor **8** (RBA = 20.5) or for the corresponding hydroxyferrocifen (RBA = 11.5),[9] which is easily explained by the presence of a phenyl group bearing the *para*-malonato-(DACH)Pt substituent, a much bulkier substituent than the malonate or the ferrocene entity. Unsurprisingly, the value found for **13**, a molecule lacking an OH group, is sharply lower (RBA = 0.5). The RBA measurements discussed above were taken on cellular extracts (cytosol) from sheep uterus. Other RBA measurements can also be taken from breast-cancer-derived cell cultures (MCF7), which contain an elevated level of the alpha form of the estradiol receptor (ERα). In this case, as in those of other triphenylethylene derivatives,[41] the RBA values obtained are approximately ten times lower than those measured in cytosol.

Study of the in vitro effects of 13, 14, and (DACH)$PtCl_2$ on hormone-dependent cell lines (MCF7 and MVLN)

The proliferative/antiproliferative effects of compounds **13**, **14**, and (DACH)$PtCl_2$ were first studied on MCF7 cells, and the results obtained after 5 days of incubation are shown in Figure 1. Complex **14**, corresponding to hydroxytamoxifen bearing the (DACH)Pt fragment, shows clear antiproliferative activity on these cells, with an IC_{50} value of 4 μM. This value is better than that found by us for (DACH)$PtCl_2$ on this cell line (IC_{50} = 6.3 μM) as well as the value reported in the literature for the oxaliplatin (IC_{50} = 7.4 μM).[30] The effect found for **13**, the nonhydroxylated complex, is weaker (IC_{50} = 14 μM) than that of **14**. Since compound **13**, with no hydroxy group, was less well recognized by the receptor, it appears that the observed effect with these complexes is essentially an antiestrogenic effect, while the antiproliferative effect observed with (DACH)$PtCl_2$

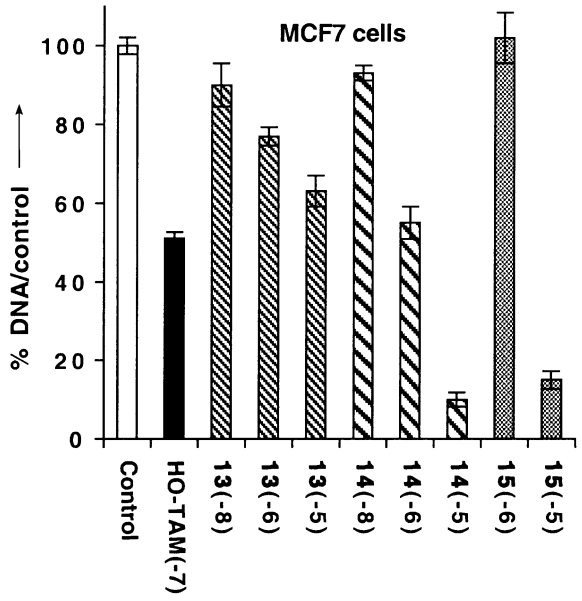


Figure 1. *Study of the effect of hydroxytamoxifen (HO-TAM), **13**, **14**, and (DACH)$PtCl_2$ (**15**) on the proliferation of MCF7 cells (estrogen-receptor-positive cells). The results are expressed as the percentage of DNA in the sample versus the DNA content of the control after 5 days of culture. Experiments were carried out in triplicate and results are shown ± limits of confidence (P = 0.1, t = 1.415). The values in brackets correspond to the log of the molarity of incubation.*

 © 2003 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

can be attributed to its known cytotoxicity. To verify this hypothesis, the effects of the compounds on MVLN cells were studied. These cells are MCF7 cells stably transfected with a reporter gene allowing expression of the firefly luciferase enzyme under the control of an estrogen response element (pVit-tk-Luc-ERE).[42] A decrease in observed luminescence (% RLU) is directly correlated with the expression of an antiestrogenic effect (inhibiting the expression of the ERE-dependent gene). The results obtained after a short period (24 h) with **13**, **14**, (DACH)$PtCl_2$, and RU 58668, a pure antiestrogen used as a reference, are shown in Figure 2. As expected,

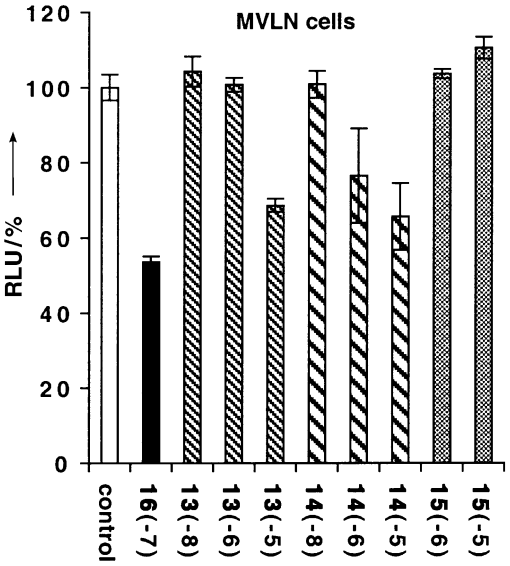


Figure 2. *Study of the antiestrogenic effect of the pure antiestrogen RU 58668 (**16**), **13, 14**, and (DACH)$PtCl_2$ (**15**) on MVLN cells (MCF7 cells stably transfected with an estrogen response element, which allows expression of the luciferase enzyme). The results are expressed as the percentage of luciferase activity (% RLU) after 24 h culture. The mean values from duplicate experiments are shown ± limits of confidence (P = 0.1, t = 1.638) except for **15** (one experiment). The values in brackets correspond to the log of the molarity of incubation.*

RU 58668 shows a marked effect, while (DACH)$PtCl_2$ has no effect on this cell line. Complex **14** has an inhibitory effect at molarities of 1 μM and 10 μM, while **13** has an effect only at high molarity (10 μM). These results confirm that the observed effect is essentially antihormonal. Addition of the (DACH)Pt entity to tamoxifen or hydroxytamoxifen produces no marked additive or synergic effect on their antiproliferative potencies, as observed in vitro on MCF7 cell lines. An interesting possibility, raised by a perceptive reviewer, is that the observed RBA values for the platinum complexes **13** and **14** may be attributable to hydrolysis products. However, the ester precursor **8** in fact shows a markedly higher RBA value (20%) than that of the platinum complex. When hydrolyzed, the diester **8** yields the corresponding dicarboxylic acid, which spontaneously loses carbon dioxide to form the monocarboxylic acid. However, there is no detectable hydrolysis under the conditions (3 h at 0 °C) at which the RBA values were measured.

Conclusion

The goal of this study was to examine the antiproliferative activity of complexes resulting from the coupling of an inorganic cytotoxic moiety—(DACH)Pt—with an antiestrogen on hormone-dependent cells lines (MCF7 and MVLN). After synthesis and characterization of nanovectors complexed with Pt, biochemical studies showed that for certain complexes, such as **14**, recognition of the estrogen receptor remained good. In addition, attachment of a vector to (DACH)Pt produced, under certain conditions, an antiproliferative effect greater than that of the uncomplexed (DACH)Pt molecule. Here it is worth considering the favorable antiestrogenic effect of the vector at concentrations in the micromolar range. At these concentrations platinum has no determining synergic effect that could give rise to a new SERM with improved therapeutic potential. This is a topic that represents a considerable current challenge, and has in fact led us to undertake the systematic study of organometallic and coordination complexes that can be delivered to specific targets, including, in the case of breast cancers, the estrogen receptors. Results have been varied, which justified a systematic approach that has proved to be full of surprises.

Examination of all the currently available data gives rise to the following differing situations:

a) The antiproliferative effect is due to the vector, and the organometallic moiety does not improve the effects of the SERM, no matter what concentration is used. In particular, this is the case for the hydroxytamoxifen derivative bearing a $CpRe(CO)_3$ group, which behaves almost identically to hydroxytamoxifen.[12] These stable species have future promise for use with radionuclides of Re and Tc.[43]

b) The effect of the organometallic moiety counteracts the antiestrogenic behaviour of the vector and produces a species with proliferative activity; this is the case with the Cp_2TiCl_2 entity, which, when attached to tamoxifen, behaves as a powerful estrogen, probably as a result of in situ release of a Ti(IV) species.[13]

c) A synergy exists between the cytotoxic organometallic moiety and its organic vector, which results in unique antiproliferative effects on breast cancer cells classed ER(+) and ER(−).[9, 10]

d) Finally, there is the case seen above, in which coupling of the antiestrogenic skeleton to a cytotoxic inorganic moiety results in a product with properties superior to those of the coordination complex alone, but the principal antiproliferative component appears to be associated with the antiestrogenic organic skeleton.

The synergy of the two components, one antiestrogenic and the other cytotoxic, is not obvious, which suggests that targets other than ERα, for example ERβ, calmodulin, protein kinase C, or growth factors, either binding antiestrogens or undergoing antiestrogenic action, may be implicated.[44, 45]

It is clear that the range of possibilities is broad, varied, and currently unpredictable. A systematic study combining chemistry and biology is the only option in the search for new SERMs with novel properties.

 © 2003 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Experimental Section

General remarks: Anhydrous THF and anhydrous diethyl ether were obtained by distillation from sodium/benzophenone. Methanol (Prolabo) was used without any further purification. Dichloro((*R*,*R*)-*trans*-1,2-diaminocyclohexane)platinum—(DACH)$PtCl_2$—was a gift from Debiopharm (Lausanne, Switzerland); all the others reagents were obtained from Aldrich. Thin layer chromatography was performed on silica gel 60 GF254. Infrared spectra were obtained on an FTIR BOMEM Michelson-100 spectrometer, ^1H and ^{13}C NMR spectra were recorded on Bruker 200 MHz and 400 MHz spectrometers, mass spectra were acquired on a Nermag R 10–10C spectrometer, and melting points were measured with a Kofler device. Elemental analyses were performed by the regional microanalysis department of the Université Pierre et Marie Curie. High resolution mass spectrometry (HRMS) was performed at the Ecole Normale Supérieure, Paris. The ketone **5** was prepared by a literature procedure.[4]

Diethyl 4-propionylphenylmalonate (2): Diethyl phenylmalonate (5.000 g, 22 mmol) and propionyl chloride (2.43 mL, 23.6 mmol) were dissolved in CH_2Cl_2 (60 mL) in a Schlenk tube purged with argon. $AlCl_3$ (8.40 g, 63 mmol) was added to the mixture in small portions (solid), with stirring. The red solution obtained was heated at reflux for 12 h, the solvent was evaporated, and the organic products were extracted with dichloromethane. After solvent removal, the crude product was purified by silica gel column chromatography with diethyl ether/pentane (3:7) as eluent to give **2** (6.080 g), isolated as a yellow oil, in 95% yield ($R_f = 0.2$, diethyl ether/pentane 8:2). ^1H NMR (200 MHz, $CDCl_3$): $\delta = 7.90$ (d, $J = 8.2$ Hz, 2H; aromatic), 7.46 (d, $J = 8.2$ Hz, 2H; aromatic), 4.64 (s, 1H; $CH(COOCH_2CH_3)_2$), 4.17 and 4.16 ($2 \times$ q, 2×2H; CH_3CH_2OCO), 2.93 (q, $J = 7.2$ Hz, 2H; CH_3CH_2), 1.20 (t, 6H; CH_3CH_2OCO), 1.18 (t, 3H; CH_3CH_2CO) ppm.

[4-(3-Dimethylamino-propyloxy)-phenyl]-4′-hydroxyphenyl-ketone (6): NaH (1.87 g) was washed with hexane (10 mL) in a Schlenk tube purged with argon. 4,4′-dihydroxybenzophenone (5.00 g, 23.36 mmol) in THF (20 mL) was added. After the mixture had been stirred for 20 min, freshly distilled DMF (50 mL) was added, followed by (3-chloropropyl)dimethylamine hydrochloride (4.00 g, 25.30 mmol) in solid form (4 portions). After the mixture had been heated at reflux for 30 min, a white precipitate of **6** had formed. The solution was concentrated and allowed to cool to room temperature, and the white precipitate obtained was isolated by filtration to give **6** (4.47 g; 64%), m.p.: 180 °C. ^1H NMR (200 MHz, CD_3OD): $\delta = 7.79$, 7.74, 7.05 and 6.95 ($4 \times$ d, 8H; aromatic rings), 4.16 (t, $J = 6.4$ Hz, 2H; $OCH_2CH_2CH_2N(CH_3)_2$), 2.42 (t, $J = 6.9$ Hz, 4H; $OCH_2CH_2CH_2N(CH_3)_2$), 2.18 (s, 6H; $OCH_2CH_2CH_2N(CH_3)_2$), 1.94 (m, 3H; $OCH_2CH_2CH_2N(CH_3)_2$) ppm; elemental analysis: calcd (%) for $C_{18}H_{21}O_3N$: C 72.22, H 7.07, N 4.68; found C 72.11, H 7.11, N 4.61.

Tamoxifen malonate derivative 7: $TiCl_4$ (4.25 mL, 38.4 mmol) was added dropwise at -10 °C to a suspension of zinc powder (1.50 g, 22.9 mmol) in THF (160 mL). A solution of ketone **2** (2.71 g, 9.24 mmol) and ketone **5** (2.80 g, 9.89 mmol) in THF (10 mL) was added dropwise, and the resulting mixture was then heated for 3 h. After cooling to room temperature, the mixture was hydrolyzed with $NaHCO_3$ solution (10%, 20 mL). After ether extraction (3×100 mL) and solvent removal, the crude product was chromatographed on a silica gel column with ethyl ether/pentane (3:7) as eluent to give **7** (2.30 g, 85%) as a white solid. M.p.: 79 °C; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.32$ (t, 2H; H_m of α ring), 7.25 (m, 1H; H_p of α ring), 7.23 (d, 2H; H_o of α ring), 7.20 (d, $J = 8.1$ Hz, 2H; H_m of β ring), 7.10 (d, $J = 8.1$ Hz, 2H; H_o of β ring), 6.74 (d, $J = 8.5$ Hz, 2H; H_o of α' ring), 6.52 (d, $J = 8.5$ Hz, 2H; H_m of α' ring), 4.52 (s, 1H; $CH(COOEt)_2$), 4.20 (q, $J = 7.1$ Hz, 4H; $OCOCH_2CH_3$), 3.85 (t, 2H; $OCH_2CH_2CH_2N(CH_3)_2$), 2.39 (t, 2H; $OCH_2CH_2CH_2N(CH_3)_2$), 2.43 (q, $J = 7.4$ Hz, 2H; CH_2CH_3), 2.22 (s, 6H; $OCH_2CH_2CH_2N(CH_3)_2$), 1.86 (qt, 2H; $OCH_2CH_2CH_2N(CH_3)_2$), 1.23 (t, $J = 7.1$ Hz, 6H; $OCOCH_2CH_3$), 0.91 (t, $J = 7.4$ Hz, 3H; CH_2CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 168.0$ (COOEt), 156.8 (C_p of α' ring), 143.6 (C_{ipso} of α ring), 142.2 (C_{ipso} of β ring), 140.5 (C=Cβ), 138.5 (Cα=C), 135.0 (C_{ipso} of α' ring), 131.7 (C_o of α' ring), 130.3 (C_p of β ring), 129.7 (C_o of β ring), 129.3 (C_m of β ring), 128.6 (C_m of α ring), 127.9 (C_o of α ring), 126.4 (C_p of α ring), 113.1 (C_m of α' ring), 65.7 ($OCH_2CH_2CH_2N(CH_3)_2$), 61.5 ($OCOCH_2CH_3$), 57.5 ($CH(COOCH_2CH_3)_2$), 56.3 ($OCH_2CH_2CH_2N(CH_3)_2$), 45.3 ($OCH_2CH_2CH_2N(CH_3)_2$), 28.8 (CH_2CH_3), 27.4 ($OCH_2CH_2CH_2N(CH_3)_2$), 13.8 ($COOCH_2CH_3$), 13.5 (CH_2CH_3) ppm; IR (KBr): $\tilde{\nu} = 1738$ cm^{-1}; MS (EI, 70 eV) *m/z*: 543 $[M]^+$, 470 $[M - COOEt]^+$; elemental analysis: calcd (%)for $C_{34}H_{41}O_5N$: C 75.11, H 7.59, N 2.57; found: C 75.09, H 7.59, N 2.48.

Hydroxytamoxifen malonate derivative (8): The preparation of this compound was analogous to that of **7**; zinc powder (0.780 g, 12 mmol), $TiCl_4$ (1.55 mL, 14 mmol), ketone **2** (1.23 g, 4.2 mmol), and ketone **6** (1.20 g, 4 mmol) yielded (after chromatography on a silica gel column with ethyl acetate/Et_3N (9:1) as eluent) **8** (1.20 g, 55%) as a white solid. ^1H NMR (200 MHz, CD_3COCD_3): $\delta = 7.27 - 7.10$ (m, 6H), 6.91 (d, $J = 8.6$ Hz, 2H), 6.73 (d, $J = 7.6$ Hz, 2H), 6.48 (d, $J = 8.6$ Hz, 2H), 4.67 (s, 1H; $CH(COOEt)_2$), 4.16 (q, $J = 7.2$ Hz, 4H; $OCOCH_2CH_3$), 4.04 (t, $J = 6.5$ Hz, 2H; $OCH_2CH_2CH_2N(CH_3)_2$), 2.52–2.40 (m, 4H; $OCH_2CH_2CH_2N(CH_3)_2$ + CH_2CH_3), 2.20 (s, 6H; $OCH_2CH_2CH_2N(CH_3)_2$), 1.99–1.89 (m, 2H; $OCH_2CH_2CH_2N(CH_3)_2$), 1.20 (t, $J = 7.12$ Hz, 6H; $OCOCH_2CH_3$), 0.91 (t, $J = 7.4$ Hz, 3H; CH_2CH_3) ppm; ^{13}C NMR (50 MHz, CD_3COCD_3): $\delta = 168.7$ (CO), 158.9, 156.3, 143.4, 140.6, 139.7, 136.9, 135.2, 132.6, 131.7, 131.2, 130.5, 129.7, 115.0 and 114.8 (aromatics + C=C), 66.7 ($OCH_2CH_2CH_2N(CH_3)_2$), 61.9 ($OCOCH_2CH_3$), 58.0 ($CH(COOCH_2CH_3)_2$), 56.8 ($OCH_2CH_2CH_2N(CH_3)_2$), 45.6 ($OCH_2CH_2CH_2N(CH_3)_2$), 28.6 ($OCH_2CH_2CH_2N(CH_3)_2$), 28.2 (CH_2CH_3), 14.3 ($OCOCH_2CH_3$), 13.9 (CH_2CH_3) ppm; MS (EI, 70 eV) *m/z*: 559 $[M]^+$, 487, 395.

Barium salt of the tamoxifen malonate derivative (11): Diester **7** (0.600 g, 1.1 mmol) was dissolved in methanol (20 mL) in a Schlenk tube purged with argon. $Ba(OH)_2 \cdot 8H_2O$ (9.8 mL of a 0.11 M solution) was added dropwise with stirring over a 3 h period, while the pH of the solution was kept below 12. The disappearance of **7** was followed by TLC and, after removal of the solvent under vacuum, the white precipitate obtained was washed with water and acetone to give **11** (0.467 g, 68%) as a white powder. IR (KBr): $\tilde{\nu}_{CO} = 1605$, 1578 cm^{-1}; elemental analysis calcd (%) for $C_{30}BaH_{31}NO_5 \cdot 3H_2O$: C 53.23, H 5.51, N 2.07; found: C 52.84, H 5.15, N 2.04.

Barium salt of the hydroxytamoxifen malonate (12): Compound **12** was prepared by the same procedure as used for the synthesis of **11**. Diester **8** (0.368 g, 0.66 mmol) and $Ba(OH)_2 \cdot 8H_2O$ (14 mL, 0.11 M solution) gave **12** (0.295 g, 70%) as a white powder. IR (KBr): $\tilde{\nu}_{CO} =$ 1606, 1575 cm^{-1}.

DACH-Pt malonate derivative of tamoxifen (13): (DACH)$PtCl_2$ (0.242 g, 0.64 mmol) was dispersed in degassed water (29 mL) in a Schlenk tube previously purged with argon and kept in the dark by wrapping the tube with aluminium foil. $AgNO_3$ (0.216 g, 1.28 mmol) was added with stirring, and the mixture was stirred at room temperature for 24 h and subsequently at 50 °C for 30 min, to give a colourless solution and a white precipitate of AgCl. After filtration, the solution obtained was added dropwise to a suspension of barium salt **11** (0.200 g, 0.32 mmol) in methanol (29 mL). After stirring overnight, the solution was concentrated to 2 mL and water was then added until a precipitate formed. The precipitate was filtered and washed with water and diethyl ether to give **13** (0.205 g, 81%) as a white powder. Dec.: 240 °C; ^1H NMR (200 MHz, DMSO-d_6): $\delta = 7.42 - 7.05$ (m, 9H),

 © 2003 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

6.80 (d, $J = 8.4$ Hz, 2H), 6.62 (d, $J = 8.6$ Hz, 2H), 5.96 and 5.27 ($2 \times$ m, 2×2H; $2 \times NH_2$), 4.50 (s, 1H), 3.89 (m, 2H), 2.60 – 2.39 (m, 4H), 2.30 (s, 6H; $OCH_2CH_2CH_2N(CH_3)_2$), 1.95 (m, 4H), 1.47 (m, 2H), 1.23 (m, 2H), 1.05 – 0.80 (5H); IR (KBr): $\tilde{\nu} = 1932$ (NH_2), 1629 (COOPt) cm^{-1}. HRMS: $C_{36}H_{46}O_5N_3{}^{195}Pt$ calcd: 795.3089; found: 795.3089 $[M+H]^+$.

DACH-Pt malonate derivative of hydroxytamoxifen (14): As in the preparation of **13**, (DACH)$PtCl_2$ (0.332 g, 0.87 mmol) and $AgNO_3$ (0.297 g, 1.75 mmol), upon treatment with barium salt **12** (0.280 g, 0.44 mmol), gave **14** (0.215 g, 61%) as a white powder. Dec.: 250 °C; ^{1}H NMR (200 MHz, DMSO-d_6): 7.20 – 6.60 (m, 8H) 6.60 (d, $J = 8.6$ Hz, 2H), 6.40 (d, $J = 8.4$ Hz, 2H), 5.96 and 5.27 ($2 \times$ m, 2×2H; $2 \times NH_2$), 4.50 (s, 1H), 3.89 (m, 2H), 2.20 – 1.80 (m, 12H), 1.20 (s, 6H; $OCH_2CH_2CH_2N(CH_3)_2$), 0.95 (m, 5H); IR (KBr): $\tilde{\nu} = 2932$ (NH_2), 1637 (C00Pt) cm^{-1}; HRMS: $C_{36}H_{46}O_6N_3{}^{195}Pt$ calcd: 811.3038; found: 811.3041 $[M+H^+]$.

Determination of the RBA values of 13 and 14 for the estrogen alpha receptor: Aliquots (200 µL) of sheep uterine cytosol, prepared as described previously[46] were incubated for 3 h at 0 °C with [6,7-^{3}H]-estradiol (2×10^{-9} M, specific activity 1.96 TBq mmol^{-1}) in the presence of nine concentrations of the hormones to be tested. At the end of the incubation period, the free and bound fractions of the tracer were separated by protamine sulfate precipitation. The percentage reduction in binding of [^{3}H]-estradiol (Y) was calculated by using the logit transformation of Y (logit Y: $\ln(Y/1-Y)$) versus the log of the mass of the competing steroid. The concentration of unlabelled steroid required to displace 50% of the bound [^{3}H]-estradiol was calculated for each steroid tested, and the results were expressed as an RBA. The RBA value of estradiol is by definition equal to 100%.

Tests on MCF7 and MVLN cells:

Culture materials: Earle's based minimal essential medium (MEM), foetal bovine serum (FBS), L-glutamine, penicillin, gentamicin, and streptomycin were obtained from Gibco (Ghent, Belgium), plastic culture materials from Falcon (Ghent, Belgium).

Culture conditions: MCF7 cells were obtained from the Michigan Cancer Foundation (Detroit) and MVLN cells (tk-vit ERE stably transfected MCF7 cells) from Pons.[42] Cells were maintained in monolayer culture in Dulbecco-MEM with 10% thermally inactivated FBS, L-glutamine (0.6 mg mL^{-1}), and a cocktail of antibiotics (gentamicin 40 µg mL^{-1}, penicillin 100 U mL^{-1}, streptomycin (100 µg mL^{-1}) added. For MCF7 cells, growth of the cells was assessed by measurement of the DNA content of treated and untreated (control) cells after 120 h of culture.[47]

ERE-dependent transcriptional activity in MVLN cells: MVLN cells (pVit-tk-Luc-ERE stably transfected in MCF7 cells) were cultured for 3 – 4 days in 35-mm diameter Falcon dishes (plating density 80 000/dish) in 10% depleted endogeneous steroid (dextran coated charcoal (DCC) treatment). Ethanolic solutions of 13 of the compounds to be tested were subsequently added to the medium and the culture pursued until the luciferase assay was carried out (24 h). For that purpose, the medium was removed and cells were washed twice with PBS buffer. A minimal volume (250 µL) of a fivefold-diluted lysis solution (Promega E 153A) was then added to the dishes, and these were maintained under mild agitation for 20 min to extract luciferase. Lysed cells were subsequently detached with a scraper (Costar 3010) and centrifuged for 5 min at 12 000 g to clarify the extracts. A 20-µL aliquot of each extract was finally mixed at room temperature with luciferase reactant medium (100 mL, Promega E15A/E152A) prepared according to the manufacturer's protocol. Induced light was measured with a Berthold luminometer (Lumat LB 9507). Induction of the luciferase was expressed in arbitrary units with respect to the light measured with a blank (RLU). To compare RLU data, the protein content of each extract was measured by the Coomassie method (PIERCE) and the data was expressed per mg protein.

[1] J. C. Allegra, M. E. Lippman, E. B. Thompson, R. Simon, A. Barlock, L. Green, K. K. Huff, H. M. Do, S. C. Aitken, *Cancer Res.* **1979**, *39*, 1447 – 1454.
[2] M. J. Clark, W. L. McGuire, C. A. Hubay, O. H. Pearson, J. S. Marshall, *N. Engl. J. Med.* **1983**, *309*, 1343 – 1347.
[3] E. R. DeSombre, P. P. Carbone, E. V. Jensen, W. L. McGuire, S. A. Wells, J. L. Wittliff, M. B. Lipsett, *N. Engl. J. Med.* **1979**, *301*, 1011 – 1012.
[4] J. C. Doré, J. Gilbert, E. Bignon, A. Crastes de Paulet, T. Ojasoo, J. F. Pons, J. P. Raynaud, J. F. Miquel, *J. Med. Chem.* **1992**, *35*, 573 – 583.
[5] V. C. Jordan, *Tamoxifen for the treatment and prevention of breast cancer*, PRR, New York, **1999**.
[6] P. R. Kym, G. M. Anstead, K. G. Pinney, S. R. Wilson, J. A. Katzenellenbogen, *J. Med. Chem.* **1993**, *36*, 3910 – 3922.
[7] S. G. Nayfield, J. E. Karp, L. G. Ford, F. A. Dorr, B. S. Kramer, *J. Natl. Cancer Inst.* **1991**, *23*, 1450 – 1459.
[8] S. Ray, A. Tandon, I. Dwivedy, S. R. Wilson, J. P. O'Neil, J. A. Katzenellenbogen, *J. Med. Chem.* **1997**, *37*, 696 – 700.
[9] G. Jaouen, S. Top, A. Vessières, G. Leclercq, J. Quivy, L. Jin, A. Croisy, *C. R. Acad. Sci., Ser. IIc: Chim.* **2000**, *3*, 89 – 93.
[10] S. Top, A. Vessières, C. Cabestaing, I. Laïos, G. Leclercq, C. Provot, G. Jaouen, *J. Organomet. Chem.* **2001**, *637 – 639*, 500 – 506.
[11] E. A. Vladusic, A. E. Hornby, F. K. Guerra-Vladusic, R. Lupu, *Cancer Res.* **1998**, *58*, 210 – 214.
[12] G. Jaouen, S. Top, A. Vessières, P. Pigeon, G. Leclercq, I. Laïos, *Chem. Commun.* **2001**, 383 – 384.
[13] S. Top, E. B. Kaloun, A. Vessières, I. Laïos, G. Leclercq, G. Jaouen, *J. Organomet. Chem.* **2002**, *643 – 644*, 350 – 356.
[14] B. Lippert, *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*, John Wiley and Sons, New York, **1999**.
[15] B. Rosenberg, L. Vancamp, J. E. Trosko, V. H. Mansour, *Nature* **1969**, *222*, 385.
[16] B. Rosenberg, *Cancer Res.* **1970**, *30*, 1799.
[17] E. von Angerer in *Metal Complexes in Cancer Chemotherapy* (Ed.: B. K. Keppler), Wiley-VCH, Weinheim (Germany), **1993**, pp. 73 – 83.
[18] C. Chesne, G. Leclercq, P. Pointeau, H. Patin, *Eur. J. Med. Chem.* **1986**, *21*, 321 – 327.
[19] J. Altman, T. Castrillo, W. Beck, G. Bernhardt, H. Schönenberger, *Inorg. Chem.* **1991**, *30*, 4085 – 4088.
[20] H. Calvert, I. Judson, W. J. F. Van der Vijgh, *Cancer Surv.* **1993**, *17*, 189 – 217.
[21] M. C. Christian, *Semin. Oncol.* **1992**, *19*, 720 – 730.
[22] L. R. Kelland, *Crit. Rev. Oncol. Hematol.* **1993**, *15*, 191 – 219.
[23] C. Meijer, N. H. Mulder, H. Timmer-Bosscha, W. J. Sluiter, G. J. Meersma, E. G. E. de Vries, *Cancer Res.* **1992**, *52*, 6885 – 6889.
[24] E. Wong, C. M. Giandomenico, *Chem. Rev.* **1999**, *99*, 2451 – 2466.
[25] S. G. Chaney, *J. Oncol.* **1995**, *6*, 1291 – 1305.
[26] Y. Kidani, K. Inagaki, M. Igo, A. Hashi, K. Kuretani, *J. Med. Chem.* **1978**, *21*, 1315.
[27] G. Mathé, Y. Kidani, M. Segiguchi, M. Eriguchi, G. Fredj, G. Peytavin, J. L. Misset, S. Brienza, F. De Vassals, D. Chenu, C. Bourut, *Biomed. Pharmacother.* **1989**, *43*, 237 – 250.
[28] P. Soulié, E. Raymond, E. Cvitkovic, S. Brienza *Bull. Cancer* **1997**, *84*, 665 – 673.
[29] T. Tashiro, Y. Kawada, S. Y. Y. Kidani, *Biomed. Pharmacother.* **1989**, *43*, 251 – 260.
[30] E. Raymond, C. Buquet-Fagot, S. Djelloul, J. Mester, E. Cvitkovic, P. Allain, C. Louvet, C. Gespach, *Anti-Cancer Drugs* **1997**, *8*, 876 – 885.
[31] P. L. Coe, C. E. Scriven, *J. Chem. Soc. Perkin Trans. 1* **1986**, 475.
[32] S. Top, B. Dauer, J. Vaissermann, G. Jaouen, *J. Organomet. Chem.* **1997**, *541*, 355 – 361.
[33] S. Gauthier, J. Mailhot, F. Labrie, *J. Org. Chem.* **1996**, *61*, 3890 – 3893.
[34] P. Bitha, S. G. Carvajal, R. V. Citarella, R. G. Child, E. F. Delos Santos, T. S. Dunne, F. E. Durr, J. J. Hlavka, S. A. Lang, H. L. Lindsey, G. O. Morton, J. P. Thomas, R. E. Wallace, Y. I. Lin, R. C. Haltiwanger, *J. Med. Chem.* **1989**, *32*, 2015 – 2020.
[35] M. Filipova-Voprsalova, J. Drobnik, B. Sramek, J. Kvetina, *J. Controlled Release* **1991**, *17*, 89 – 98.
[36] O. Gandolfi, H. C. Apfelbaum, J. Blum, *Inorg. Chim. Acta* **1987**, *135*, 27 – 31.

 © 2003 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

[37] A. R. Khokhar, I. H. Krakoff, M. P. Hacker, J. J. McCormack, *Inorg. Chim. Acta* **1985**, *108*, 63 – 66.
[38] A. H. Talebian, D. Bensely, P. S. Schein, D. Green, *Anti-Cancer Drug Des.* **1990**, *5*, 371 – 380.
[39] E. R. Jamieson, S. J. Lippard, *Chem. Rev.* **1999**, *99*, 2467 – 2498.
[40] S. K. Mauldin, M. Plescia, F. A. Richard, S. D. Wyrick, R. D. Voyksner, S. G. Chaney, *Biochem. Pharmacol.* **1988**, *37*, 3321 – 3333.
[41] S. Stoessel, G. Leclercq, *J. Steroid Biochem.* **1986**, *25*, 677.
[42] M. Pons, D. Gagne, J. C. Nicolas, M. Mehtali, *BioTechniques* **1990**, *9*, 450 – 459.
[43] A. Duatti, *Technetium, Rhenium and Other Metals* in *Chemistry and Nuclear Medicine 4 and 5*, S. G. Editoriali, Padova, **1999** and **2000**.
[44] R. Clarke, F. Leonessa, J. N. Welch, T. C. Skaar, *Pharmacol. Rev.* **2001**, *53*, 25 – 71.
[45] A. A. Colletta, J. R. Benson, M. Baum, *Breast Cancer Res. Treat.* **1994**, *31*, 5.
[46] A. Vessières, S. Top, A. A. Ismail, I. S. Butler, M. Loüer, G. Jaouen, *Biochemistry* **1988**, *27*, 6659 – 6666.
[47] G. Leclercq, N. Devleeschouwer, J. C. Heuson, *J. Steroid Biochem.* **1983**, *19*, 75 – 85.

Received: December 31, 2002
Revised version: April 15, 2003 [F 550]

 © 2003 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim