Eur J Med Chem (1992) 27, 301–305

Estrogen-*cis*-dichloroethylenediamineplatinum (II) complexes: synthesis and evaluation of binding affinity for estrogen receptors and the effect on breast cancer MCF-7 cells

DM Spyriounis[1], VJ Demopoulos[1], PN Kourounakis[1]*, D Kouretas[2], A Kortsaris[2], O Antonoglou[2]

[1]*Department of Pharmaceutical Chemistry, School of Pharmacy, University of Thessaloniki, 540 06 Thessaloniki;* [2]*Research Department, Theagenion Cancer Hospital, Thessaloniki, Greece*

(Received 28 November 1990; accepted 7 October 1991)

estrogen-*cis*-dichloroethylenediamineplatinum (II) complexes / estrogen receptor binding / cancer cell growth inhibition

Introduction

Numerous efforts have been made to increase the selectivity of chemotherapeutics towards cancer cells and decrease their systemic toxicity. In this respect, a possibility for selectivity is offered by hormone-dependent tumors, such as certain breast cancers, which selectively concentrate estrogens [1].

In order to induce cytotoxic effects towards hormone-dependent tumor cells, chemotherapeutic groups have been attached to carrier ligands by diverse synthetic approaches [2–9]. A prerequisite for specificity of these novel cytotoxic agents is a sufficient binding of the drug to the estrogen receptor, which will allow the selective uptake into the hormone sensitive cells [10]. Recently, it has been proposed that spacer chains attached to estrogens or antiestrogens could allow relatively bulky cytotoxic groups to be transported *in vivo* while ensuring minimal interference between the carrier moiety and the steroidal binding sites [8, 9, 11]. However, this type of substitution, connecting estrogens with a *cis*-dichlorodiamineplatinum (II) group *via* a spacer chain, has not yet been described to the best of our knowledge.

In this study, the synthesis of ethylenediamine derivatives of estrone (**1b**) and estradiol (**2b**) as well as their *cis*-dichloroplatinum (II) complexes (**1c** and **2c**) is described. The RBA (relative binding affinity compared to estradiol = 100) of these new compounds to estrogen receptors was evaluated, and the most potent was further studied for cytotoxic activity against breast cancer MCF-7 cells.

Chemistry

Estrogen derivatives **1b** and **2b** were synthesized by displacement of bromine with 1,2-ethanediamine in **1a** and **2a**. Crucial factors for a successful reaction were both the choice of the reaction solvent (MeOH) and the careful control of the reaction temperature (*ca*, 50°C).

Efforts to synthesize **1a** from estrone (**1**), sodium hydroxide and 1,3-dibromopropane in DMSO [12] resulted in a complex mixture of products. Alternatively, the phase transfer catalyzed technique was adopted under a variety of conditions. In the initial experiments a major by-product was the allyl ether **1d**, resulting in low yields of isolated bromopropyl ether **1a**. Finally, by using tetrahydrofuran as the solvent, benzyltributylammonium chloride [13] or TDA-1 [14] as the phase transfer catalysts and a gradual addition of a certain excess of sodium hydroxide, good yields of **1a** were attained. At the same time, relatively small amounts of the elimination by-product **1d** were formed. Tetrahydrofuran is not normally used as a solvent for phase transfer catalyzed reactions. However, the mechanism of the

*Correspondence and reprints

above described reaction probably involves a phase transfer process, because in the absence of catalyst no reaction takes place. This new procedure seems of general applicability for alkylating estrone. Thus, 3-methoxyestrone (**1e**) was isolated in quantitative yields by using dimethyl sulfate as the alkylating agent.

On the other hand, efforts to etherify estradiol (**2**) to yield **2a** under phase transfer conditions similar to those described above were unsuccessful, resulting in isolation of only starting materials. Thus, **2a** was

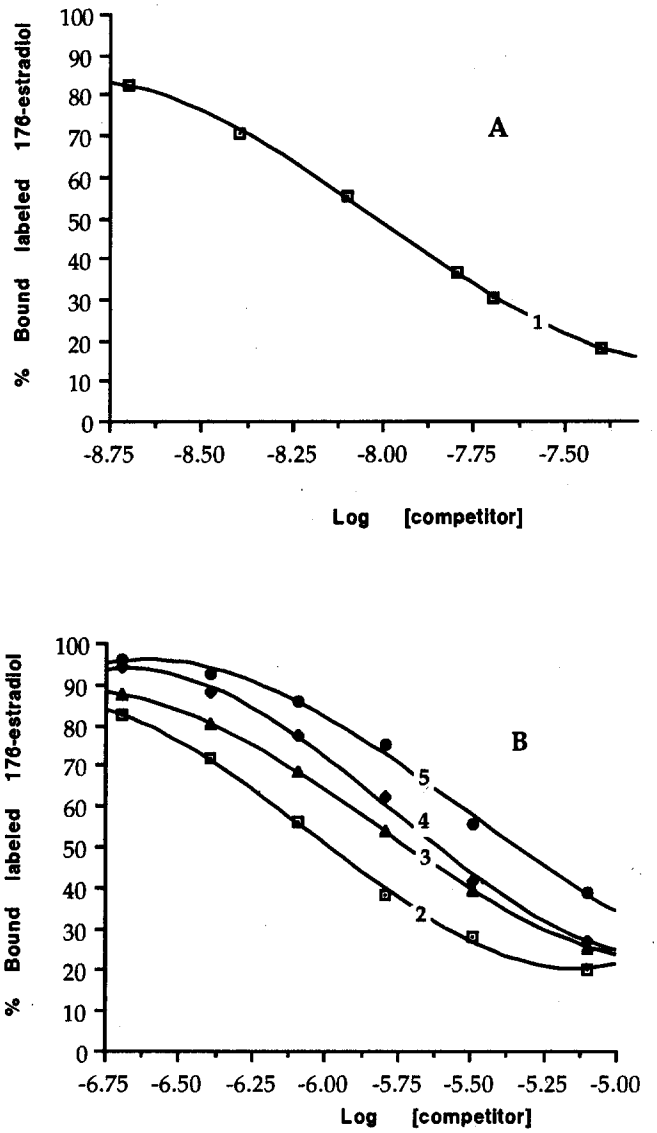
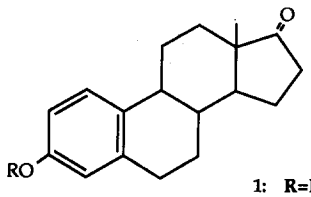
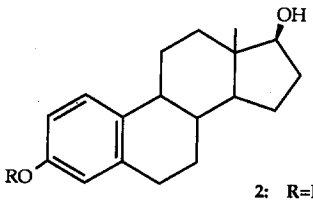


Fig 1. Competitive binding assay for estrogen receptor labeled with 17β-[^{3}H]estradiol of estrane derivatives. **A.** unlabeled 17β-estradiol (1). **B.** **1c** (2), **2c** (3), **1b** (4), **2b** (5).



1: R=H
1a: R=$CH_2CH_2CH_2Br$
1b: R=$CH_2CH_2CH_2NHCH_2CH_2NH_2.2HCl$
1c: R=$CH_2CH_2CH_2NHCH_2CH_2NH_2.PtCl_2$
1d: R=$CH_2CH{=}CH_2$
1e: R=CH_3



2: R=H
2a: R=$CH_2CH_2CH_2Br$
2b: R=$CH_2CH_2CH_2NHCH_2CH_2NH_2.2HCl$
2c: R=$CH_2CH_2CH_2NHCH_2CH_2NH_2.PtCl_2$

synthesized by reducing **1a** with $NaBH_3CN$ in a mixture of MeOH–tetrahydrofuran.

Hydride reductions of D-ring keto steroids are known to proceed with selective hydride delivery to the α-side [15]. Furthermore, the chemical shift for 18-CH_3 in the ^{1}H NMR of **2a** is in accord with the expected selectivity of the carbonyl reduction [16]. Therefore, the configuration of 17-OH in **2a** is assigned as β.

The complexation of diamine **1b** was carried out in H_2O and of diamine **2b** in a mixture of H_2O–tetrahydrofuran, with the addition of an aqueous solution of potassium tetrachloroplatinate. The pH of the reaction was monitored and adjusted to 6.5 with a 0.1 N NaOH solution, until the end of the reaction [7]. The *cis*-configuration of the platinum complexes, thus formed, was indicated by strong absorption in the far infrared close to 320 cm^{-1} of the Pt–Cl bonds [17].

Biological results and discussion

The binding affinities for the estrogen receptors of both the diamino-ligands and the platinum complexes were measured in a competitive binding assay with 17β-[^{3}H]estradiol. Breast tumor cytosol was used as a receptor source [18] and the dextran-coated charcoal

(DCC) method was used for binding affinity assay [8]. The displacement curves of bound 17β-[^{3}H]estradiol by the tested compounds are shown in figure 1. The relative binding affinities (RBA) were calculated as the ratio of the molar concentration of the 17β-estradiol and steroid required to decrease the receptor-bound radioactivity by 50% multiplied by 100.

It was found that the diamino-ligands **1b** and **2b** and the corresponding platinum complexes **1c** and **2c** were able to displace estradiol from its receptor. Compound **1b** exhibited RBA 0.43 and its platinum complex **1c** 0.98. Contrary to our expectations, based on the established SAR of estrogens [6], reduction of the carbonyl group in position C-17 leads to compounds with lower binding affinity for the estrogen receptor. Thus, compound **2b** exhibited RBA 0.26 and the corresponding platinum complex **2c** 0.57.

Furthermore, it was noted that the employed buffer (TEA) for the receptor preparation does not have a decisive effect on the binding affinity of complex **1c**. This derives from the fact that the competing curves of **1c** in various buffer systems are not significantly different (fig 2).

A prerequisite for specificity of these new agents is a sufficient binding to the estrogen receptor. Calculations on the basis of the number of receptors per cell (≈ 1 000–10 000) and the possible drug concentration show that the RBA value should be at least 1% of that of estradiol [10]. Estrone-*cis*-dichloroethylenediamineplatinum (**II**) complex **1c**, with RBA of 0.98, closely approximates this limit.

Preliminary experiments showed that compound **1c** (5 μM) produced a 71% growth inhibition of hormone-dependent breast cancer MCF-7 cell line. *cis*-DDP, used as a reference, showed 76% inhibition at the same concentration (lit [3] value = 68%). Further experiments are underway for the determination of a possible selectivity of **1c** between hormone-dependent and non-hormone-dependent cell lines.

Experimental protocols

Chemical methods

Melting points are uncorrected and were determined in a Carl Zeiss, Jena hot-plate apparatus. Infrared spectra were recorded with a Perkin–Elmer 597 spectrophotometer, and nuclear magnetic resonance spectra were recorded with a Bruker AW-80 spectrometer with internal tetramethylsilane reference. Elemental analyses were performed on a Perkin–Elmer 240 automated analyzer. Flash chromatography [19] was carried out using Merck 9385 silica gel, and thin-layer chromatography on Merck 5554 silica gel on aluminum sheets. Petroleum ether refers to the fraction bp 40–60°C.

3-[(3-Bromopropyl)oxy]estra-1,3,5(10)-trien-17-one ***1a***

To a stirred and under a nitrogen atmosphere mixture of estrone (**1**) (1.0 g, 3.7 mmol), 1,3-dibromopropane (1.2 g, 5.95 mmol) and benzyltributylammonium chloride (0.1 g, 0.32 mmol) in tetrahydrofuran (70 ml) powdered NaOH (0.32 g, 8 mmol) was added in portions over a period of 24 h. This reaction was conducted under a nitrogen atmosphere. The solvent was evaporated under reduced pressure (bath t < 30°C) and the residue was flash-chromatographed on silica gel with ether–petroleum ether (1:10 → 1:1) as the eluent to afford in this order: a), 3-[(3-allyl)oxy]estra-1,3,5(10)-trien-17-one **1d**, 0.21 g (18%). Recrystallization from ether-petroleum ether afforded an analytical sample, mp = 98–100°C, TLC: R_f =0.51 (ether-petroleum ether 1:1); IR (KBr): 1730, 1675, 1645, 1610, 1245 cm^{-1}; NMR ($CDCl_3$) δ 6.60–7.30(m, 3H, C-1-H, C-2-H and C-4-H); 5.75–6.30(m, 1H, CH=C); 5.10–5.50(m, 2H, $C=CH_2$); 4.30–4.50(m, 2H, OCH_2); 2.80 (m, 2H, C-6-H); 0.50–2.60(m, remaining steroid H). Anal calcd for $C_{21}H_{26}O_2$: C, 81.25; H, 8.44. Found: 81.35; 8.61. b), 3-[(3-bromopropyl)-oxy]estra-1,3,5(10)-trien-17-one **1a**, 0.9 g (62%). Recrystallization from ether–petroleum ether afforded an analytical sample, mp = 89–90°C, TLC: R_f = 0.44 (ether–petroleum ether 1:1); IR (KBr): 1730, 1605, 1285, 1235 cm^{-1}; NMR ($CDCl_3$) δ 6.40–7.20(m, 3H, C-1-H, C-2-H and C-4-H); 4.00 (t, 2H, OCH_2); 3.50 (t, 2H, CH_2Br); 2.75 (m, 2H, C-6-H); 0.80 (s, 3H, 18-CH_3); 1.10–2.60 (m, remaining steroid H). Anal calcd for $C_{21}H_{27}O_2Br$: C, 64.65; H, 6.95. Found: C, 64.34; H, 6.91.

3-[[3-(Aminoethylamino)propyl]oxy]estra-1,3,5(10)-trien-17-one dihydrochloride salt ***1b***

A solution of **1a** (0.3 g, 0.77 mmol) and 1,2-ethanediamine (0.24 g, 3.99 mmol) in MeOH (20 ml) was heated at 50°C (internal temperature) for 7 h under a nitrogen atmosphere. At the end of this period, the solvent was removed under reduced pressure, the residue was partitioned between water and CH_2Cl_2, the organic phase washed with water, dried (K_2CO_3) and concentrated to a small volume. Acidification with ethereal HCl resulted in the formation of a white solid which was

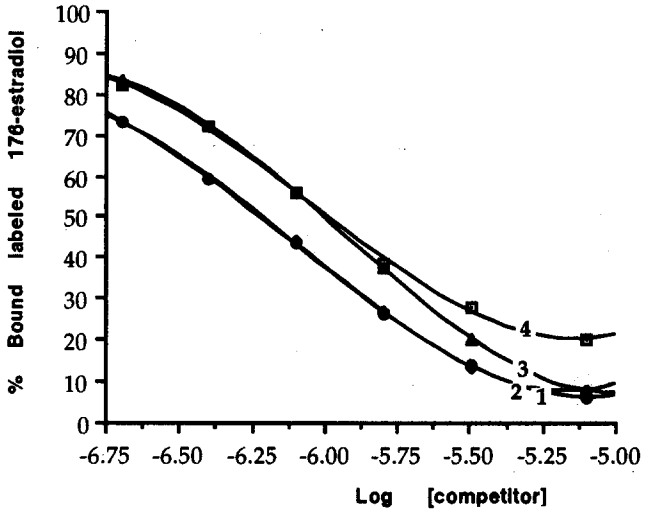


Fig 2. Competitive binding assay for estrogen receptor labeled with 17β-[^{3}H]estradiol of complex **1c** in: acetate buffer (1), phosphate buffer (2), acetate buffer supplemented with NaCl (0.06 M) (3), TEA buffer (4).

collected and washed with ether (0.27 g, 79%). Recrystallization from MeOH–ether afforded an analytical sample, mp = 168–170°C (dec); IR (KBr) 3500–3300, 1730, 1605, 1240 cm^{-1}; NMR (DMSO–d_6 + CF_3COOD): δ 8.30–8.70 and 9.40–9.80 (m, 5H, ^+NH); 6.45–7.25 (m, 3H, C-1-H, C-2-H and C-4-H); 3.95 (t, 2H, OCH_2); 2.90–3.90(m, 6H, CH_2N); 2.80 (m, 2H, C-6-H); 0.50–2.20 (m, remaining steroid H). Anal calcd for $C_{23}H_{37}N_2O_2Cl_2$: C, 62.15; H, 8.39; N, 6.30. Found: C, 61.88; H, 8.34; N, 6.45.

cis-Dichloro[estra-1,3,5(10)-trien-17-on-3-[oxopropyl-3-(aminoethylamino)]]-platinum(II) **1c**

To a solution of **1b** (0.15 g, 0.34 mmol) in distilled water (10 ml), a solution of potassium tetrachloroplatinate (0.14 g, 0.34 mmol) in distilled water (5 ml) was added with stirring. The pH of the reaction was monitored (and maintained) to 6.5. After 4 h the resulting precipitate was filtered off, washed several times with water and dried to afford 0.07 g (32%) of product, mp = 219–220°C; IR (KBr): 3600–3000, 1725, 1605, 1240, 318 cm^{-1}. Anal calcd for $C_{23}H_{35}N_2O_2Cl_2Pt$: C, 43.33; H, 5.53; N, 4.39. Found: C, 43.11; H, 5.68; N, 4.32.

3-[(3-Bromopropyl)oxy]estra-1,3,5(10)-trien-17β-ol **2a**

Compound **1a** (0.45 g, 1.12 mmol) and a trace of bromocresol green were dissolved in a mixture of methanol (6.5 ml) and tetrahydrofuran (4.5 ml) and then $NaBH_3CN$ (0.25 g, 3.87 mmol) was added. The solution immediately turned deep blue, and an ethereal solution of HCl was added dropwise with stirring to restore the yellow color. The solution was stirred for 1 h while the yellow color was maintained with occasional additions of ethereal HCl. The volatiles were evaporated under reduced pressure and the residue was directly flash-chromatographed on silica gel with ether as the eluent to afford 0.45 g (99%) of product as a viscous oil; NMR ($CDCl_3$) δ 6.45–7.30 (m, 3H, C-1-H, C-2-H and C-4-H); 4.10 (t, 2H, OCH_2); 3.40–3.85 (m, 3H, CH_2Br and C-17-H); 2.80(m, 2H, C-6-H); 0.75 (s, 3H, 18-CH_3); 1.10–2.60 (m, remaining steroid H).

3-[[3-(Aminoethylamino)propyl]oxy]estra-1,3,5(10)-trien-17β-ol dihydrochloride salt **2b**

Treatment of a solution of **2a** (0.32 g, 0.79 mmol) in methanol (22 ml) with 1,2-ethanediamine (0.24 g, 3.99 mmol) as described above for the preparation of **1b** afforded 0.15 g (41%) of a white solid which was recrystallized from methanol-ether to afford an analytical sample, mp = 115–119°C; IR (KBr): 3500–3200, 1605, 1205 cm^{-1}; NMR (DMSO–d_6 + CF_3COOD) δ 8.30–8.90 and 9.60–10.00 (m, 5H, ^+NH), 6.55–7.30 (m, 3H, C-1-H, C-2-H and C-4-H); 5.70 (s, lH, 17-OH); 4.00(m, 3H, OCH_2 and C-17-H); 3.00–3.60(m, 6H, CH_2N); 2.80 (m, 2H, C-6-H); 0.50–2.70 (m, remaining steroid H). Anal calcd for $C_{22}H_{39}N_2O_2Cl_2$: C, 61.87; H, 8.80; N, 6.27. Found: C, 62.11; H, 8.91; N, 6.32.

cis-Dichloro[estra-1,3,5(10)-trien-17β-ol-3-[oxopropyl-3-(aminoethylamino)]]platinum(II) **2c**

Treatment of a solution of **2b** (0.2 g, 0.45 mmol) in a mixture of tetrahydrofuran (4 ml) and distilled water (1 ml) with a solution of potassium tetrachloroplatinate (0.187 g, 0.45 mmol) in distilled water (9 ml) as described above for the preparation of **1c** afforded 0.22 g (77%) of product, mp = 268–270°C (dec); IR (KBr): 3600–3000, 1605, 1248, 318 cm^{-1}. Anal calcd for $C_{23}H_{37}N_2O_2Cl_2Pt$: C, 43.19; H, 5.83; N, 4.38. Found: C, 42.89; H, 5.75; N, 4.28.

3-Methoxyestra-1,3,5(10)-trien-17-one **1e**

A mixture of estrone (**1**) (0.5 g, 1.8 mmol) and powdered NaOH (0.2 g, 5.0 mmol) in tetrahydrofuran (35 ml) was stirred for 12 h under a nitrogen atmosphere. To the resulting mixture, benzyltributylammonium chloride (0.037 g, 0.12 mmol) was added and the stirring was continued for 12 h while dimethyl sulfate (0.25 ml, 3.12 mmol) was gradually added. At the end of this period the unreacted dimethyl sulfate was destroyed with the addition of 0.5 ml concentrated aqueous ammonium hydroxide solution and the solvents were removed under reduced pressure. The residue was partitioned between water and CH_2Cl_2. The organic phase was washed with water, dried (K_2CO_3) and the volatiles evaporated under reduced pressure. The solid residue was recrystallized from ether–petroleum ether to afford 0.5 g (95%) of the product, mp = 175–176°C (lit [20] mp = 168–169°C); NMR ($CDCl_3$) δ 6.60–7.30 (m, 3H, C-1-H, C-2-H and C-4-H); 3.70 (s, 3H, OCH_3); 2.82 (m, 2H, C-6-H); 0.50–2.60(m, remaining steroid H). Anal calcd for $C_{19}H_{24}O_2$: C, 80.24; H, 8.50. Found: C, 79.89; H, 8.69.

Biological methods

Estradiol receptor binding assay

[2,4,6,7,16,17-^{3}H]-17β-Estradiol (164 Ci/mmol) was obtained from Amersham. Cytosols, obtained from human breast tumor tissues [18] containing receptors in levels over 1000 fmol/mg protein, were used as estrogen receptor preparations. TEA [Tris–HCl (0.01 M/0.006 M, pH 7.5) supplemented with EDTA (0.001 M), sucrose (0.25 M) and 1,4-dithiothreitol (0.5 mM)] was used as a buffer.

The relative binding affinity (RBA) of the drug for the estrogen receptors was measured according to a competition method. The 300 μl incubation mixture comprised 5 nM [^{3}H]-17β-estradiol (added in 50 μl of TEA), 50 μl of competing ligand (dissolved in aqueous ethanol or DMSO solutions) and 200 μl of cytosol. Ethanol or DMSO in the incubation mixture was < 1%. The mixture was incubated for 30 min at 4°C, and then 1 ml of dextran-coated charcoal (DCC) slurry (1.0% charcoal Norit A and 0.1% dextran T32 in TEA) was added to the tubes, the contents were mixed and then centrifuged at 3 500 *g* for 10 min to pellet the charcoal. The radioactivity of 300 μl supernatant sample was measured by liquid scintillation. Nonspecific binding was calculated with 500 nM diethylstilbestrol as competitor. Six concentrations of competitor (1 nM to 10 μM) were chosen to provide values between 10–90% of specifically bound radioactivity. Experiments were performed in triplicate (SD < 10%). Radioactivity was plotted as a function of the log concentration of competing ligand in the assay (fig 1). The RBA was calculated as the ratio of the molar concentration of estradiol and test compound required to decrease the amount of bound radioactivity by 50%, multiplied by 100.

Complex **1c** was also tested according to the above described procedure using instead the following buffer systems: a), phosphate buffer (0.025 M, pH 7.3) supplemented with sucrose (0.25 M) and 1,4-dithiothreitol (0.5 mM); b), acetate buffer (0.025 M, pH 7.3) supplemented with sucrose (0.25 M) and 1,4-dithiothreitol (0.5 mM); c), acetate buffer (0.025 M, pH 7.3) supplemented with NaCl (0.06 M), sucrose (0.25 M) and 1,4-dithiothreitol (0.5 mM). Results are shown in figure 2.

Effect of complex **1c** *on growth of MCF-7 cell line*

For the assay a previously described procedure [3] was applied with modifications. Human breast carcinoma MCF-7 cells (derived from metastatic pleural effusion) were cultivated as monolayers in Earle's based minimal essential medium supplemented with 10% fetal calf serum and 10 μg/ml insulin. Cells, growing in log phase, were suspended in trypsin solution

(0.25% trypsin and 0.02% EDTA in saline), diluted in medium, 2 ml of the cell suspension (containing ≈ 10^5 cells) plated into 35 x 10 mm tissue culture dishes and incubated in a humidified 95% air 5% CO_2 atmosphere. After 16 h, the drug (**1c** dissolved in 0.5% aqueous DMSO solution, *cis*-DDP in Dulbecco's phosphate buffered saline (PBS)) was added at a volume of 20 µl and the cultures were incubated for 3 h. The drug treatment was terminated by aspiration of the medium, washing of the cell layer with 2 ml PBS and adding 2 ml of drug-free medium. The cells were cultivated for 78 h, then resuspended in trypsin solution and counted in a hemocytometer. The viability of the cells counted was assessed using the tryptan blue exclusion method. Experiments were performed in quadruplicate (SD < 20%).

References

1 Raus J, Martens H, Leclercq G (1980) *In: Cytotoxic Estrogens in Hormone Receptive Tumors*. Academic Press, London
2 Hamacher H, Christ E (1983) *Arzneim Forsch/Drug Res* 33, 347–352
3 Gandolfi O, Blum J, Mandelbaum-Shavit F (1984) *Inorg Chim Acta* 91, 257–261
4 Wappes B, Jennerwein M, vonAngerer E, Schönenberger H, Engel J, Berger M, Wrobel KH (1984) *J Med Chem* 27, 1280–1286
5 Delbarre A, Oberlin R, Roques BP, Borgna JL, Rochefort H, Le Pecq JB, Jacquemin-Sablon A (1985) *J Med Chem* 28, 752–761
6 Chesne C, Leclercq G, Pointeau P, Patin H (1986) *Eur J Med Chem* 21, 321–327
7 Georgiadis MP, Haroutounian SA, Chondros KP (1987) *Inorg Chim Acta* 138, 249–252
8 Knebel N, vonAngerer E (1988) *J Med Chem* 31, 1675–1679
9 Krohn K, Kulikowski K, Leclercq G (1989) *J Med Chem* 32, 1532–1538
10 Katzenellenbogen JA, Katzenellenbogen BS (1982) *Breast Cancer Res Treat* 2, 347–353
11 DaSilva JN, vanLier JE (1990) *J Med Chem* 33,430–434
12 Johnston RAW, Rose ME (1979) *Tetrahedron* 35, 2169–2173
13 McKillop A, Fiaud JC, Hug RP (1974) *Tetrahedron* 30, 1379–1382
14 Soula G (1985) *J Org Chem* 50, 3717–3721
15 Dryden NL (1972) *In: Organic Reactions in Steroid Chemistry* (Fried J, Edwards JA, eds) Van Nostrad Reinhold, NY, vol 1, 1–60,
16 Horwitz JP, Iyer VK, Vardhan HB, Corombos J, Brooks SC (1986) *J Med Chem* 29, 692–698
17 Müller R, Gust R, Jennerwein M, Reile H, Laske R, Krischke W, Bernhardt G, Spruss T, Engel J, Schönenberger H (1989) *Eur J Med Chem* 24, 341–348
18 Clark JH, Peck Jr. EJ, Schrader WT, O'Malley BW (1976) *In: Methods in Cancer Research* (Busch H, ed) Academic Press, NY, vol 12, 367–417
19 Still WC, Khan M, Mitra A (1978) *J Org Chem* 43, 2923–2925
20 Butenandt A, Störmer I, Westphal U (1932) *Z Physiol Chem* 208, 149–154