Bioorganic & Medicinal Chemistry 20 (2012) 6969–6978

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and cytotoxic activities of estrone and estradiol *cis*-dichloroplatinum(II) complexes

Miroslav Kvasnica [a,*], Lucie Rarova [b], Jana Oklestkova [c], Milos Budesinsky [a], Ladislav Kohout [a]

[a] Institute of Organic Chemistry and Biochemistry ASCR, Flemingovo n. 2, 16610 Prague 6, Czech Republic
[b] Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Šlechtitelů 11, 78371 Olomouc, Czech Republic
[c] Laboratory of Growth Regulators, Institute of Experimental Botany ASCR & Palacky University, Šlechtitelů 11, 78371 Olomouc, Czech Republic

ARTICLE INFO

Article history:
Received 26 July 2012
Revised 11 October 2012
Accepted 14 October 2012
Available online 22 October 2012

Keywords:
Steroids
Estrone
Estradiol
Platinum complex
Cytotoxicity

ABSTRACT

Sixteen platinum(II) complexes of estrone and estradiol were synthesized in this work to evaluate their cytotoxic activity against several cancer cell lines including estrogen dependent and independent ones. The synthesis of all the complexes was done in three steps. The reaction of steroids with dibromoalkanes was followed by a reaction of the bromoalkyl steroids with 2-(aminomethyl)pyridine or 2-(2-aminoethyl)pyridine. The last step was a reaction of steroidal diamino ligands with potassium tetrachloroplatinate to obtain the desired platinum(II) complexes. Cytotoxicity assays showed that most of the complexes prepared are active against the cancer cell lines used—CEM, U-2 OS, MCF7, MCF7 AL, MDA-MB-468, BT-474, BT-549, and BJ fibroblasts. The six-membered platinum complexes are more active than five-membered ones.

1. Introduction

Platinum complexes are still widely used as anticancer drugs. Since the discovery[1,2] of antiproliferative activity of cisplatin in 1965, many platinum compounds with a significant anticancer effect have been prepared.[3–15] Some of them are used clinically in the treatment of solid tumors nowadays (Fig. 1).[16,17] However, their toxicity causes occasional undesirable side effects and certain tumors develop resistance, which has motivated research groups worldwide to synthesize more specific analogues. One of the possibilities to solve the problem of specificity is using steroidal hormones (e.g., estrogens, androgens) as a transporter of platinum into cancer cells. Steroidal units have often been chosen with respect to possible translocation into the nucleus of mammary tumor cells by the steroid hormone receptor system, especially in hormone-dependent cancer (e.g., breast cancer).[18–20] Estrone (**1**) and estradiol (**2**) play an important role in the evolution and development of hormone dependent breast cancers in more than 90% of cases.[21] This can be caused primarily by the total cumulative exposure of the breast tissue to bioavailable estrogens.[22] This fact sparked an interest in the preparation and structure–activity relationship studies of new cytotoxic estrogen derivatives. Furthermore, over the last few decades many anticancer estrogen derivatives have been synthesized[23–30] while utilizing two potential anticancer units—platinum and estrogens. Many steroidal platinum complexes have been prepared up to now.[31–41]

Herein, we report on the synthesis of aminomethylpyridine and aminoethylpyridine derivatives of estrone and estradiol as well as their *cis*-dichloroplatinum complexes. While most of the estrogen platinum complexes were prepared using other position than C-3, in this work we studied the anticancer activity of complexes bound to phenolic hydroxyl group despite the fact that the free phenolic hydroxyl is essential for receptor affinity.[42] All the platinum(II) complexes were tested against several cancer cell lines, T-lymphoblastic leukemia CEM, human osteosarcoma U-2 OS, estrogen-dependent human breast adenocarcinoma MCF7 (ER+), estrogen dependent human breast adenocarcinoma cultivated in the steroid free medium MCF7 AL (ER+), estrogen-independent human breast adenocarcinoma MDA-MB-468 (ER−), an ERα (−) and ERβ (+) human breast ductal carcinoma cell line BT-474, estrogen-independent human breast ductal carcinoma BT-549 (ER−) and against normal human fibroblasts (BJ).

2. Results and discussion

2.1. Synthesis and characterization of the complexes

The synthesis of 16 platinum(II) complexes **19a**–**26a** and **19b**–**26b** was done in three reaction steps starting with estrone and estradiol (Scheme 1). Both starting steroids were used for the reaction with dibromoalkanes in the presence of sodium hydroxide to

* Corresponding author. Tel.: +420 220 183 316; fax: +420 220 183 578.
E-mail address: kvasnica@uochb.cas.cz (M. Kvasnica).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.10.013

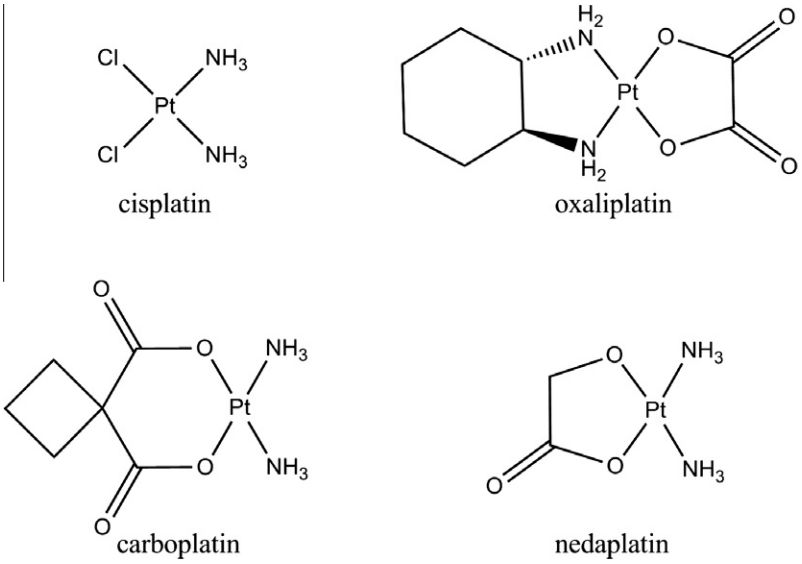


Figure 1. Structure of clinically used platinum complexes.

afford bromoalkyl derivatives **3**–**10**. Such bromoderivatives were then used for the reaction with 2-(aminomethyl)pyridine or 2-(2-aminoethyl)pyridine in ethanol to prepare steroidal diaminoligands **11**–**18**. These ligands were used for a complexation reaction with potassium tetrachloroplatinate in a solution of DMF and water.

All of the compounds synthesized were fully characterized with ^{1}H NMR spectra, IR spectra, MS, and elemental analysis. Due to their low solubility in basic non-complexing NMR solvents ($CDCl_3$, CD_3OD, dioxane-d_8, benzene-d_6), compounds **19a**, **20a**, **21a**, **22a**, and **24a** were not characterized by NMR spectra, but other techniques (IR, MS and elemental analysis) were sufficient to prove their structure and purity.

In contrast to free ligands, the IR spectra of the platinum complexes showed a significant shift of the NH_2 band to lower frequencies by about 100–200 cm^{-1}. However, a more significant shift is caused by the use of a different method of analysis for platinum complexes where chloroform was replaced with potassium bromide because of their low solubility in chloroform. Other vibrations like the C=O (~1730 cm^{-1}), O–H (~3600 cm^{-1}) or C=C of aromatic systems (~1610, 1570 cm^{-1}) are strong but without any significant change when compared with the free ligands.

The ^{1}H NMR spectra of the above platinum complexes showed significant downfield shifts of pyridyl moiety hydrogens, especially H-6 (Δ ~0.7 ppm). Because of the formation of a new ring, aliphatic CH hydrogens lost their chemical equivalence, which resulted in two separate peaks in contrast to one peak in the case of free ligands. Moreover, in some cases it is possible to see splitting of several signals in ^{13}C NMR (see Supplementary data). This is caused by formation of a chiral center at the aliphatic amino group after platinum complexation.

2.2. Stability assay

It was necessary to test the stability of the platinum complexes to be sure that these compounds are not decomposed during cytotoxicity experiments. Dulbecco's Modified Eagle's Medium (DMEM) was used for the stability test, because this medium was utilized for the 72 h cultivation of cancer cells. After incubation (see 4.46. in the Section 4), the mixture was analyzed by HPLC (High Performance Liquid Chromatography). Six measurements were done according to the time of incubation (3, 4, 5, 24, 48, 72 h). To test the stability of the complexes, we also analyzed each fraction by MS (ESI). According to the MS spectra, no free ligand was detected; consequently, there is no decomplexation process in the medium used. However, we observed a decrease of the peak intensity. This can be caused by the hydrolysis of chloride ions or complexation of the whole complex to another component of the medium, most probably to sulfur-containing molecules. Nevertheless, these processes do not affect the cytotoxicity assays as they are quite common for dichloroplatinum complexes in physiological solutions and blood.

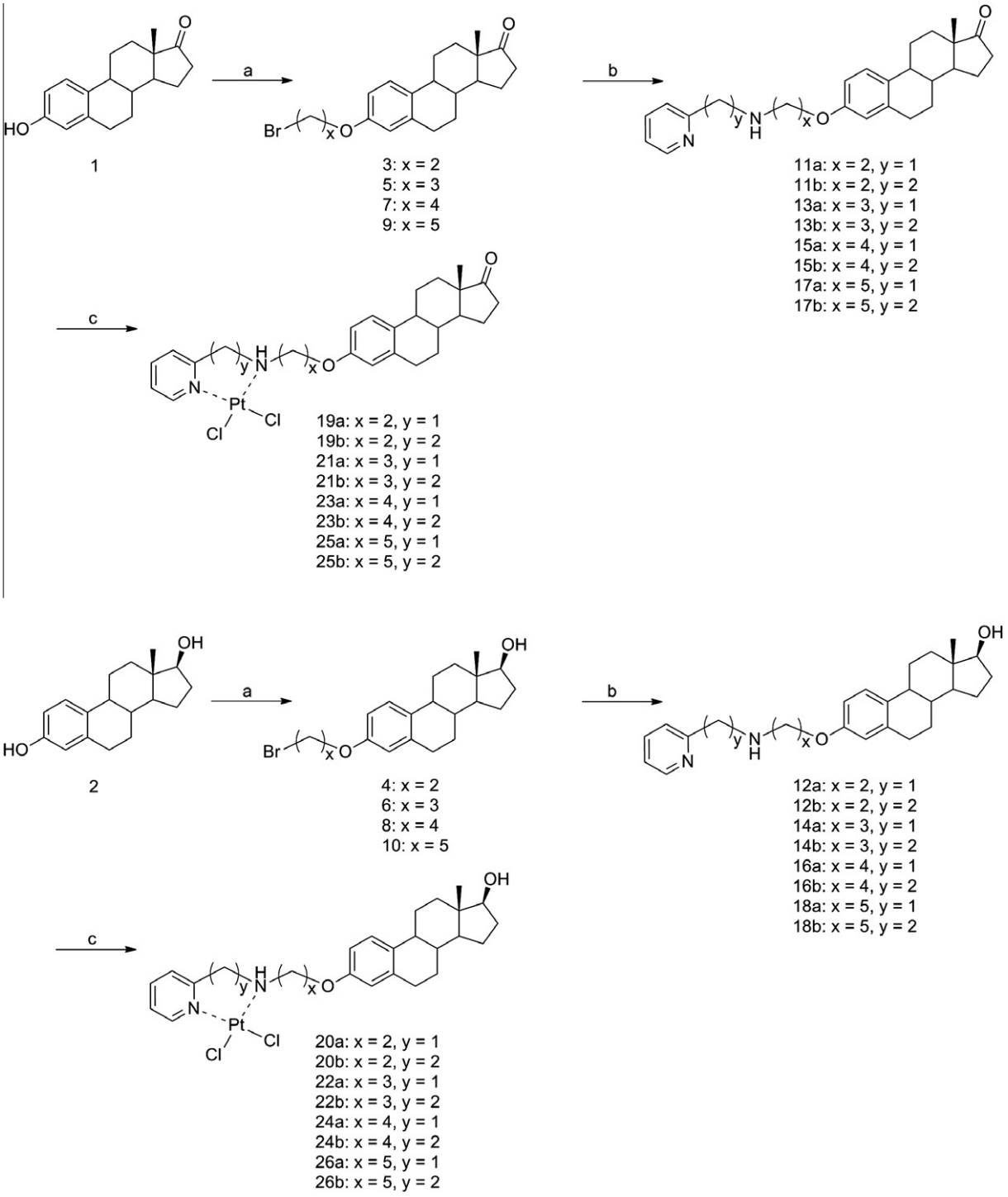
2.3. Cytotoxicity

The platinum complexes were screened against various tumor cells. The suspension cell line CEM proved to be the most sensitive to the tested compounds (Table 1); all of the compounds tested were effective against it at micromolar concentrations. The cytotoxicity (IC_{50}) value of **25b** (1.2 μM) was highest in the CEM cells, which was comparable with the activity of cisplatin; the cytotoxicity of some complexes against MCF7 (ER+) and MDA-MB-468 (ER−) cells was 3- to 4-fold better than cisplatin.

Estrone 6-membered ring complexes were more cytotoxic towards all of the cell lines than the other tested complexes. The compounds were also tested for cytotoxicity to normal BJ human fibroblasts and all proved to be less toxic towards them than towards cells of the malignant CEM cell line (Table 1). The length of the side chain seems to be optimal at $x = 2$ for 17β-estradiol 5-membered platinum ring derivatives, where we observed IC_{50} of 8.1 and 7.4 μM for the MCF7 AL (ER+) and MDA-MB-468 (ER−) cells, respectively. The complexes with longer side chains ($x = 3$–5) were less active.

The IC_{50} values of estrone and 17β-estradiol were also included for comparison. The platinum(II) complexes were more effective on all tested cell lines than estrone, or 17β-estradiol. The effects of estrone and 17β-estradiol on the growth of MCF7 (ER+) and MDA-MB-330 (ER−) breast-cancer cells had been determined previously after 72 h.[43] 17β-Estradiol inhibited the growth of both breast cancer cell lines in concentrations >50 μM. Estrone did not have any significant influence on the growth of breast cancer cells.[43]

However, it was important to indicate that all of the platinum(II) complexes were generally more cytotoxic than cisplatin on breast cancer cell lines.[44]



Scheme 1. Reagents: (a) $(CH_2)_xBr_2$, NaOH, THF, H_2O; (b) 2-(aminomethyl)pyridine or 2-(2-aminoethyl)pyridine, EtOH; (c) $K_2[PtCl_4]$, DMF, H_2O.

Our results show that there is no significant difference between estrogen-dependent (e.g., MCF7 (ER+), BT-474 (ER+)), and estrogen-independent (e.g., MDA-MB-468 (ER−), BT-549 (ER−)) breast cancer cell lines (Table 1) which confirms the necessity of free hydroxyl group on position 3.

3. Conclusion

Within the global research in the field of anti-tumor agents, a number of platinum(II) complexes were studied. Our study demonstrates that new platinum(II) complexes of steroidal diamines can result in compounds with significant cytotoxic activity against tumor cell lines of different histogenetic origin, especially breast-cancer tumors. These complexes showed cytotoxicity also against the human fibroblast BJ, selected as an example of normal cells. This fact proves general knowledge that substitution in position 3 leads to loss of receptor selectivity. However, all of the compounds tested were more cytotoxic in cancer cells than in normal human cells—fibroblasts. The synthesized complexes are also stable against the decomplexation of platinum from steroidal ligands, which is very important for further studies of their biological activities and for the future design of other analogues.

Table 1
IC_{50} (μM) values for the complexes **19a**–**26a** and **19b**–**26b** and estrone, 17β-estradiol and cisplatin as controls obtained from the Calcein AM assays with the tested cancer cell lines and normal human fibroblasts after 72 h; means ± SD obtained from three independent experiments performed in triplicates. Data for cisplatin are taken from the literature.[35,44] NT = not tested.

		CEM	U-2OS	MCF7(ER+)	MCF7 AL(ER+)	BT 474 (ER+)	BT 549(ER−)	MDA-MB-468(ER−)	BJ
ESTRONE	**19a**	24 ± 0.1	6.2 ± 0.4	9.3 ± 1.0	7.1 ± 0.4	5.3 ± 0.3	4.9 ± 1.8	4.6 ± 0.8	6.6 ± 0.3
5-Membered ring	**21a**	3.0 ± 0.1	13.8 ± 0.1	13.2 ± 3.8	24.7 ± 0.8	12.0 ± 1.7	7.5 ± 1.0	45.1 ± 6.2	7.5 ± 0.4
	23a	2.7 ± 0.4	7.3 ± 0.0	9.7 ± 14	15.6 ± 6.2	8.0 ± 0.1	7.4 ± 1.0	22.7 ± 4.4	6.6 ± 0.2
	25a	2.2 ± 0.1	7.5 ± 0.9	9.7 ± 2.0	11.4 ± 4.2	10.1 ± 2.8	6.7 ± 0.8	13.9 ± 1.6	9.6 ± 3.9
ESTRONE	**19b**	2.5 ± 0.2	6.0 ± 0.5	7.9 ± 0.8	5.3 ± 0.1	2.8 ± 0.3	3.1 ± 0.3	5.0 ± 1.5	6.8 ± 0.6
6-Membered ring	**21b**	1.7 ± 0.6	5.2 ± 0.4	2.9 ± 0.6	6.0 ± 1.1	3.5 ± 0.1	2.6 ± 0.3	4.0 ± 1.0	2.4 ± 0.3
	23b	1.9 ± 0.3	4.9 ± 0.7	4.2 ± 1.0	6.3 ± 0.9	4.3 ± 0.7	3.9 ± 0.4	5.1 ± 0.5	3.1 ± 0.6
	25b	1.2 ± 0.1	3.0 ± 0.3	3.4 ± 0.6	5.0 ± 1.0	3.1 ± 0.1	2.5 ± 0.3	3.4 ± 1.0	2.5 ± 0.1
17β-ESTRADIOL	**20a**	4.3 ± 1.1	6.9 ± 0.4	23.5 ± 4.9	8.1 ± 0.5	8.8 ± 1.5	7.4 ± 1.3	7.4 ± 1.3	7.5 ± 0.5
5-Membered ring	**22a**	4.9 ± 1.6	8.1 ± 1.6	10.5 ± 2.9	20.9 ± 6.4	14.6 ± 7.1	8.6 ± 1.9	40.1 ± 1.5	7.0 ± 3.5
	24a	3.4 ± 1.5	8.4 ± 0.6	14.8 ± 1.2	30.4 ± 3.5	13.3 ± 6.5	8.5 ± 2.2	31.7 ± 10.8	7.6 ± 2.9
	26a	2.7 ± 0.7	14.7 ± 0.0	10.5 ± 1.5	24.5 ± 15.4	10.0 ± 7.3	7.5 ± 0.6	42.8 ± 0.5	9.5 ± 2.9
17β-ESTRADIOL	**20a**	2.2 ± 0.0	6.0 ± 0.4	7.1 ± 0.1	5.4 ± 0.4	2.8 ± 0.2	7.5 ± 1.6	7.5 ± 1.6	7.4 ± 0.7
6-Membered ring	**22b**	1.8±0.4	6.3 ± 0.5	4.1 ± 0.9	6.9 ± 0.6	5.0 ± 1.1	3.4 ± 0.1	6.9 ± 0.6	3.2 ± 0.2
	24b	2.0 ± 0.1	7.5 ± 0.6	5.6 ± 1.8	7.2 ± 0.6	3.7 ± 1.1	2.7 ± 0.1	10.5 ± 3.7	4.2 ± 1.8
	26b	1.9 ± 0.2	4.3 ± 1.3	5.0 ± 0.5	5.1 ± 1.3	4.5 ± 1.6	3.3 ± 0.1	13.4 ± 0.6	3.0 ± 0.6
ESTRONE	**1**	35.7 ± 9.7	>50	>50	>50	>50	>50	41.9 ± 4.6	>50
17β-ESTRADIOL	**2**	25.2 ± 6.4	32.4 ± 1.6	>50	>50	45.7 ± 3.7	48.4 ± 2.3	21.2 ± 0.3	>50
CISPLATIN		1.6 ± 0.5	NT	18.2 ± 0.4	NT	NT	NT	17.2 ± 2.3	5.1 ± 0.2

The additional advantage of the steroidal platinum complexes above is also the potential of the use of many modified steroids to increase the cytotoxic activity and simultaneously decrease the toxicity. Hence, this kind of complexes represents an interesting class of compounds for further studies. The synthesis of new steroid analogues of similar platinum complexes with free phenolic hydroxyl are planned for next research.

4. Experimental

4.1. General methods

The melting points were determined on a Hund H 600 apparatus (Helmut Hund, Germany). The elemental analyses (C, H, N) were carried out on a Perkin-Elmer 2400 II elemental analyzer. Optical rotations were measured on an Autopol IV polarimeter (Rudolf Research Analytical, Flanders, USA) at 25 °C in chloroform (unless otherwise stated) and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g. The infrared spectra were recorded on a Bruker IFS 55 spectrometer in chloroform or in KBr in the case of some platinum complexes. The wave numbers are given in cm^{-1}. The ^1H NMR spectra were taken in $CDCl_3$ on a Bruker AVANCE-400 (at 400 MHz) instrument with tetramethylsilane as an internal reference, unless otherwise stated. Chemical shifts are given in ppm (δ-scale), coupling constants (J) in Hz. All of the values were obtained by first-order analysis. The mass spectra (ESI) were obtained with a LTQ Orbitrap XL (Thermo Fisher Scientific). For column chromatography, neutral silica gel (60 μm) was used (Fluka). The HPLC system consisted of an Agilent 1200 Series high pressure quaternary pump, thermostatted autosampler, thermostatted column compartment, vacuum degasser, diode array detector, and a Polymer Laboratories light scattering detector. HPLC was controlled with Agilent Chemstation for the LC and LC/MS Systems. The analytical column was Agilent ZORBAX Eclipse XDB C18.

The estrone and estradiol used in this work were purchased from Steraloids, Inc. The potassium tetrachloroplatinate, 2-(aminomethyl)pyridine, 2-(2-aminoethyl)pyridine, and dibromoalkanes were purchased from Sigma–Aldrich.

4.2. General procedure for the preparation of bromoalkyl derivatives (A)

Dibromoalkane (5 mmol) was added to a solution of estrone or estradiol (3.7 mmol) and NaOH (5 mmol) in THF (20 mL) and water (5 mL). The reaction mixture was heated under reflux for 6–7 h. The end of the reaction was monitored by TLC (toluene/Et_2O 5:1). The mixture was diluted with toluene and extracted with 10% aqueous HCl, water, and dried over $MgSO_4$. The solvents were evaporated and the crude product was purified by chromatography on silica gel (toluene/Et_2O 30:1). All compounds were obtained as white crystals after crystallization from methanol.

4.3. 3-(2-Bromoethoxy)estra-1,3,5(10)-trien-17-one (3)

Compound **3** was prepared from **1** according to the general procedure A. Yield: 84%, $[\alpha]_D$ +120° (*c* 0.41). ^1H NMR ($CDCl_3$) δ 0.91 (s, 3H, CH_3), 2.25 (m, 1H, H-9), 2.39 (m, 1H, H-11α), 2.50 (dd, 1H, *J* = 19.2, *J́* = 8.8 Hz, H-16β), 2.83–2.96 (m, 2H, H-6α, H-6β), 3.62 (t, 2H, *J* = 6.4 Hz, CH_2Br), 4.27 (t, 2H, *J* = 6.4 Hz, CH_2O), 6.66 (d, 1H, *J* = 2.5 Hz, H-4), 6.67 (dd, 1H, *J* = 8.6, *J́* = 2.5 Hz, H-2), 7.21 (d, 1H, *J* = 8.6 Hz, H-1). IR *ν* (cm^{-1}) 1734, 1609, 1575, 1498, 647. MS (ESI) *m*/*z* 378 (M^+, 94), 376 (M^+, 100). Anal. Calcd for $C_{20}H_{25}BrO_2$: C, 63.66; H, 6.68. Found: C, 63.57; H, 6.73%.

4.4. 3-(2-Bromoethoxy)estra-1,3,5(10)-trien-17β-ol (4)

Compound **4** was prepared from **2** according to the general procedure A. Yield: 88%, mp 90–92 °C, $[\alpha]_D$ +59° (*c* 0.33). ^1H NMR ($CDCl_3$) δ 0.78 (s, 3H, CH_3), 1.70 (m, 1H, H-15α), 1.88 (m, 1H, H-7β), 1.95 (dt, 1H, *J* = 12.4, *J́* = 3.2 Hz, H-12β), 2.08–2.22 (m, 2H, H-9, H-16α), 2.31 (m, 1H, H-11α), 2.79–2.90 (m, 2H, H-6α, H-6β), 3.62 (t, 2H, *J* = 6.4 Hz, CH_2Br), 3.73 (t, 1H, *J* = 8.4 Hz, H-17α), 4.26 (t, 2H, *J* = 6.4 Hz, CH_2O), 6.64 (d, 1H, *J* = 2.5 Hz, H-4), 6.71 (dd, 1H, *J* = 8.6, *J́* = 2.5 Hz, H-2), 7.21 (d, 1H, *J* = 8.6 Hz, H-1). IR *ν* (cm^{-1}) 3614, 1608, 1574, 1498, 647. MS (ESI) *m*/*z* 380 (M^+, 93), 378 (M^+, 100). Anal. Calcd for $C_{20}H_{27}BrO_2$: C, 63.33; H, 7.17. Found: C, 63.30; H, 7.26%.

4.5. 3-(3-Bromopropoxy)estra-1,3,5(10)-trien-17-one (5)

Compound **5** was prepared from **1** according to the general procedure A. Yield: 86%, mp 89–92 °C, $[\alpha]_D$ +110° (*c* 0.33). ^1H NMR ($CDCl_3$) δ 0.91 (s, 3H, CH_3), 2.25 (m, 1H, H-9), 2.30 (pentet, 2H, *J* = 6.0 Hz, $BrCH_2CH_2$), 2.40 (m, 1H, H-11α), 2.51 (dd, 1H, *J* = 18.9, *J́* = 8.5 Hz, H-16β), 2.84–2.97 (m, 2H, H-6α, H-6β), 3.60 (t, 2H, *J* = 6.4 Hz, CH_2Br), 4.08 (t, 2H, *J* = 5.8 Hz, CH_2O), 6.66 (d, 1H, *J* = 2.7 Hz, H-4), 6.72 (dd, 1H, *J* = 8.7, *J́* = 2.7 Hz, H-2), 7.20 (d, 1H,

J = 8.7 Hz, H-1). IR ν (cm^{-1}) 1733, 1609, 1574, 1499, 649. MS (ESI) m/z 392 (M^+, 92), 390 (M^+, 100). Anal. Calcd for $C_{21}H_{27}BrO_2$: C, 64.45; H, 6.95. Found: C, 64.48; H, 7.05%.

4.6. 3-(3-Bromopropoxy)estra-1,3,5(10)-trien-17β-ol (6)

Compound **6** was prepared from **2** according to the general procedure A. Yield: 87%, mp 88–90 °C, $[\alpha]_D$ +61° (c 0.30). ^1H NMR ($CDCl_3$) δ 0.78 (s, 3H, CH_3), 1.70 (m, 1H, H-15α), 1.88 (m, 1H, H-7β), 1.95 (dt, 1H, J = 12.4, J' = 3.0 Hz, H-12β), 2.08–2.22 (m, 2H, H-9, H-16α), 2.31 (m, 1H, H-11α), 2.30 (pentet, 2H, J = 6.0 Hz, $BrCH_2CH_2$), 2.79–2.91 (m, 2H, H-6α, H-6β), 3.60 (t, 2H, J = 6.7 Hz, CH_2Br), 3.73 (t, 1H, J = 8.4 Hz, H-17α), 4.07 (t, 2H, J = 6.1 Hz, CH_2O), 6.64 (d, 1H, J = 2.7 Hz, H-4), 6.71 (dd, 1H, J = 8.5, J' = 2.7 Hz, H-2), 7.21 (d, 1H, J = 8.5 Hz, H-1). IR ν (cm^{-1}) 3613, 1608, 1574, 1499, 649. MS (ESI) m/z 394 (M^+, 94), 392 (M^+, 100). Anal. Calcd for $C_{21}H_{29}BrO_2$: C, 64.12; H, 7.43. Found: C, 64.03; H, 7.48%.

4.7. 3-(4-Bromobutoxy)estra-1,3,5(10)-trien-17-one (7)

Compound **7** was prepared from **1** according to the general procedure A. Yield: 87%, mp 112–113 °C, $[\alpha]_D$ +115° (c 0.38). ^1H NMR ($CDCl_3$) δ 0.91 (s, 3H, CH_3), 2.25 (m, 1H, H-9), 2.40 (m, 1H, H-11α), 2.50 (dd, 1H, J = 18.8, J' = 8.7 Hz, H-16β), 2.84–2.96 (m, 2H, H-6α, H-6β), 3.49 (t, 2H, J = 6.7 Hz, CH_2Br), 3.97 (t, 2H, J = 6.0 Hz, CH_2O), 6.64 (d, 1H, J = 2.5 Hz, H-4), 6.70 (dd, 1H, J = 8.8, J' = 2.5 Hz, H-2), 7.20 (d, 1H, J = 8.8 Hz, H-1). IR ν (cm^{-1}) 1733, 1609, 1573, 1499, 647. MS (ESI) m/z 406 (M^+, 94), 404 (M^+, 100). Anal. Calcd for $C_{22}H_{29}BrO_2$: C, 65.18; H, 7.21. Found: C, 65.11; H, 7.28%.

4.8. 3-(4-Bromobutoxy)estra-1,3,5(10)-trien-17β-ol (8)

Compound **8** was prepared from **2** according to the general procedure A. Yield: 89%, mp 108–109 °C, $[\alpha]_D$ +52° (c 0.36). ^1H NMR ($CDCl_3$) δ 0.78 (s, 3H, CH_3), 1.70 (m, 1H, H-15α), 1.85–1.97 (m, 4H, H-7β, H-12β, $BrCH_2CH_2$), 2.02–2.11 (m, 2H, CH_2CH_2O), 2.09–2.22 (m, 2H, H-9, H-16α), 2.31 (m, 1H, H-11α), 2.78–2.91 (m, 2H, H-6α, H-6β), 3.48 (t, 2H, J = 6.8 Hz, CH_2Br), 3.73 (t, 1H, J = 8.4 Hz, H-17α), 3.97 (t, 2H, J = 6.0 Hz, CH_2O), 6.62 (d, 1H, J = 2.8 Hz, H-4), 6.69 (dd, 1H, J = 8.4, J' = 2.8 Hz, H-2), 7.20 (d, 1H, J = 8.4 Hz, H-1). IR ν (cm^{-1}) 3614, 1608, 1573, 1499, 648. MS (ESI) m/z 408 (M^+, 95), 406 (M^+, 100). Anal. Calcd for $C_{22}H_{31}BrO_2$: C, 64.86; H, 7.67. Found: C, 64.80; H, 7.76%.

4.9. 3-(5-Bromopentoxy)estra-1,3,5(10)-trien-17-one (9)

Compound **9** was prepared from **1** according to the general procedure A. Yield: 91%, mp 85–87 °C, $[\alpha]_D$ +110° (c 0.33). ^1H NMR ($CDCl_3$) δ 0.91 (s, 3H, CH_3), 2.25 (m, 1H, H-9), 2.40 (m, 1H, H-11α), 2.50 (dd, 1H, J = 19.0, J' = 8.6 Hz, H-16β), 2.84–2.96 (m, 2H, H-6α, H-6β), 3.44 (t, 2H, J = 6.8 Hz, CH_2Br), 3.94 (t, 2H, J = 6.5 Hz, CH_2O), 6.64 (d, 1H, J = 2.8 Hz, H-4), 6.71 (dd, 1H, J = 8.6, J' = 2.8 Hz, H-2), 7.19 (d, 1H, J = 8.6 Hz, H-1). IR ν (cm^{-1}) 1733, 1609, 1573, 1499, 647. MS (ESI) m/z 420 (M^+, 93), 418 (M^+, 100). Anal. Calcd for $C_{23}H_{31}BrO_2$: C, 65.87; H, 7.45. Found: C, 65.79; H, 7.53%.

4.10. 3-(5-Bromopentoxy)estra-1,3,5(10)-trien-17β-ol (10)

Compound **10** was prepared from **2** according to the general procedure A. Yield: 92%, mp 79–81 °C, $[\alpha]_D$ +68° (c 0.26). ^1H NMR ($CDCl_3$) δ 0.78 (s, 3H, CH_3), 1.70 (m, 1H, H-15α), 1.80 (m, 2H, $BrCH_2CH_2$), 1.85–1.97 (m, 4H, H-7β, H-12β, CH_2CH_2O), 2.07–2.22 (m, 2H, H-9, H-16α), 2.32 (m, 1H, H-11α), 2.78–2.91 (m, 2H, H-6α, H-6β), 3.43 (t, 2H, J = 6.8 Hz, CH_2Br), 3.73 (t, 1H, J = 8.4 Hz, H-17α), 3.94 (t, 2H, J = 6.3 Hz, CH_2O), 6.62 (d, 1H, J = 2.8 Hz, H-4), 6.69 (dd, 1H, J = 8.4, J' = 2.8 Hz, H-2), 7.19 (d, 1H, J = 8.4 Hz, H-1). IR ν (cm^{-1}) 3614, 1608, 1573, 1499, 643. MS (ESI) m/z 422 (M^+, 94), 420 (M^+, 100). Anal. Calcd for $C_{23}H_{33}BrO_2$: C, 65.55; H, 7.89. Found: C, 65.49; H, 7.96%.

4.11. General procedure for the preparation of diaminoderivatives (B)

2-(Aminomethyl)pyridine or 2-(2-aminoethyl)pyridine (1.0 mmol) was added to a solution of bromoalkylestrone or bromoalkylestradiol (0.5 mmol) in EtOH (5 ml). The reaction mixture was heated under reflux for 7–8 h. The end of the reaction was monitored by TLC (toluene/Et_2O 5:1) for disappearance of the starting material. The solvent was evaporated and the crude oil product was purified by chromatography on silica gel ($CHCl_3$ with NH_3/MeOH 20:1).

4.12. 3-(2-(Pyridin-2-ylmethylamino)ethoxy)estra-1,3,5(10)-trien-17-one (11a)

Compound **11a** was prepared as a pale yellow oil from **3** according to the general procedure B using 2-(aminomethyl)pyridine. Yield: 79%, $[\alpha]_D$ +109° (c 0.25). ^1H NMR (MeOD) δ 0.90 (s, 3H, CH_3), 2.21 (m, 1H, H-9), 2.38 (m, 1H, H-11α), 2.47 (dd, 1H, J = 19.2, J' = 8.8 Hz, H-16β), 2.78–2.87 (m, 2H, H-6α, H-6β), 2.97 (t, 2H, J = 5.3 Hz, $NHCH_2CH_2$), 3.94 (s, 2H, $PyrCH_2NH$), 4.04 (t, 2H, J = 5.3 Hz, CH_2O), 6.63 (d, 1H, J = 2.8 Hz, H-4), 6.69 (dd, 1H, J = 8.5, J' = 2.8 Hz, H-2), 7.15 (d, 1H, J = 8.5 Hz, H-1), 7.29 (ddd, 1H, J = 7.7, J' = 4.8, J'' = 0.8 Hz, H_{pyr}-5), 7.46 (br d, 1H, J = 7.9 Hz, H_{pyr}-3), 7.79 (td, 1H, J = 7.7, J' = 1.6 Hz, H_{pyr}-4), 8.49 (ddd, 1H, J = 4.8, J' = 1.6, J'' = 0.8 Hz, H_{pyr}-6). IR ν (cm^{-1}) 3320, 1733, 1608, 1593, 1572, 1499. MS (ESI) m/z 405 $[M+H]^+$. Anal. Calcd for $C_{26}H_{32}N_2O_2$: C, 77.19; H, 7.97; N, 6.92. Found: C, 77.13; H, 7.98; N, 6.90%.

4.13. 3-(2-(2-(Pyridin-2-yl)ethylamino)ethoxy)estra-1,3,5(10)-trien-17-one (11b)

Compound **11b** was prepared as a pale yellow oil from **3** according to the general procedure B using 2-(2-aminoethyl)pyridine. Yield: 77%, $[\alpha]_D$ +100° (c 0.26). ^1H NMR (MeOD) δ 0.89 (s, 3H, CH_3), 2.20 (m, 1H, H-9), 2.37 (m, 1H, H-11α), 2.46 (dd, 1H, J = 19.2, J' = 8.8 Hz, H-16β), 2.77–2.86 (m, 2H, H-6α, H-6β), 2.98–3.08 (m, 6H, $PyrCH_2CH_2NHCH_2$), 4.03 (t, 2H, J = 5.2 Hz, CH_2O), 6.60 (d, 1H, J = 2.5 Hz, H-4), 6.66 (dd, 1H, J = 8.6, J' = 2.5 Hz, H-2), 7.15 (d, 1H, J = 8.6 Hz, H-1), 7.24 (ddd, 1H, J = 7.6, J' = 4.8, J'' = 1.1 Hz, H_{pyr}-5), 7.31 (br d, 1H, J = 7.7 Hz, H_{pyr}-3), 7.73 (td, 1H, J = 7.7, J' = 1.9 Hz, H_{pyr}-4), 8.43 (ddd, 1H, J = 4.8, J' = 1.6, J'' = 0.8 Hz, H_{pyr}-6). IR ν (cm^{-1}) 3317, 1733, 1608, 1594, 1571, 1499. MS (ESI) m/z 419 $[M+H]^+$. Anal. Calcd for $C_{27}H_{34}N_2O_2$: C, 77.48; H, 8.19; N, 6.69. Found: C, 77.42; H, 8.26; N, 6.75%.

4.14. 3-(2-(Pyridin-2-ylmethylamino)ethoxy)estra-1,3,5(10)-trien-17β-ol (12a)

Compound **12a** was prepared as a pale yellow oil from **4** according to the general procedure B using 2-(aminomethyl)pyridine. Yield: 81%, $[\alpha]_D$ +90° (c 0.20). ^1H NMR ($CDCl_3$) δ 0.77 (s, 3H, CH_3), 1.69 (m, 1H, H-15α), 1.83–1.96 (m, 2H, H-7β, H-12β), 2.08–2.21 (m, 2H, H-9, H-16α), 2.30 (m, 1H, H-11α), 2.78–2.87 (m, 2H, H-6α, H-6β), 3.06 (t, 1H, J = 5.2 Hz, $NHCH_2CH_2$), 3.72 (t, 1H, J = 8.4 Hz, H-17α), 4.00 (s, 2H, $PyrCH_2NH$), 4.09 (t, 2H, J = 5.2 Hz, CH_2O), 6.64 (d, 1H, J = 2.4 Hz, H-4), 6.71 (dd, 1H, J = 8.6, J' = 2.4 Hz, H-2), 7.17 (ddd, 1H, J = 7.3, J' = 5.0, J'' = 1.1 Hz, H_{pyr}-5), 7.19 (d, 1H, J = 8.6 Hz, H-1), 7.35 (br d, 1H, J = 7.5 Hz, H_{pyr}-3), 7.65 (td, 1H, J = 7.5, J' = 1.6 Hz, H_{pyr}-4), 8.56 (ddd, 1H, J = 5.0, J' = 1.6, J'' = 0.8 Hz, H_{pyr}-6). IR ν (cm^{-1}) 3614, 3320, 1607, 1593, 1572, 1499. MS (ESI)

m/*z* 407 $[M+H]^+$. Anal. Calcd for $C_{26}H_{34}N_2O_2$: C, 76.81; H, 8.43; N, 6.89. Found: C, 76.77; H, 8.48; N, 6.87%.

4.15. 3-(2-(2-(Pyridin-2-yl)ethylamino)ethoxy)estra-1,3,5(10)-trien-17β -ol (12b)

Compound **12b** was prepared as a pale yellow oil from **4** according to the general procedure B using 2-(2-aminoethyl)pyridine. Yield: 80%, $[\alpha]_D$ +50° (*c* 0.20). ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H, CH_3), 1.69 (m, 1H, H-15α), 1.93 (td, 1H, *J* = 11.9, *J́* = 3.2, H-7β), 2.07–2.21 (m, 2H, H-9, H-16α), 2.30 (m, 1H, H-11α), 2.79–2.88 (m, 2H, H-6α, H-6β), 2.99–3.12 (m, 6H, Pyr$CH_2CH_2NHCH_2$), 3.72 (t, 1H, *J* = 8.4 Hz, H-17α), 4.05 (t, 2H, *J* = 5.5 Hz, CH_2O), 6.61 (d, 1H, *J* = 2.4 Hz, H-4), 6.68 (dd, 1H, *J* = 8.4, *J́* = 2.4 Hz, H-2), 7.13 (ddd, 1H, *J* = 7.5, *J́* = 5.0, *J́* = 1.2 Hz, H_{pyr}-5), 7.18 (m, 2H, H-1, H_{pyr}-3), 7.60 (td, 1H, *J* = 7.5, *J́* = 1.9 Hz, H_{pyr}-4), 8.53 (ddd, 1H, *J* = 5.0, *J́* = 1.6, *J́* = 0.8 Hz, H_{pyr}-6). IR *ν* (cm^{-1}) 3613, 3317, 1607, 1594, 1571, 1499. MS (ESI) *m*/*z* 421 $[M+H]^+$. Anal. Calcd for $C_{27}H_{36}N_2O_2$: C, 77.10; H, 8.63; N, 6.66. Found: C, 77.02; H, 8.72; N, 6.62%.

4.16. 3-(3-(Pyridin-2-ylmethylamino)propoxy)estra-1,3,5(10)-trien-17-one (13a)

Compound **13a** was prepared as a pale yellow oil from **5** according to the general procedure B using 2-(aminomethyl)pyridine. Yield: 82%, $[\alpha]_D$ +82° (*c* 0.33). ^{1}H NMR ($CDCl_3$) δ 0.91 (s, 3H, CH_3), 2.25 (m, 1H, H-9), 2.39 (m, 1H, H-11α), 2.50 (dd, 1H, *J* = 19.2, *J́* = 8.8 Hz, H-16β), 2.79–2.91 (m, 4H, H-6α, H-6β, NHCH_2CH_2), 3.95 (s, 2H, PyrCH_2NH), 4.04 (t, 2H, *J* = 6.1 Hz, CH_2O), 6.65 (d, 1H, *J* = 2.8 Hz, H-4), 6.71 (dd, 1H, *J* = 8.5, *J́* = 2.8 Hz, H-2), 7.17 (ddd, 1H, *J* = 7.6, *J́* = 4.8, *J́* = 0.8 Hz, H_{pyr}-5), 7.19 (d, 1H, *J* = 8.5 Hz, H-1), 7.31 (br d, 1H, *J* = 7.9 Hz, H_{pyr}-3), 7.64 (td, 1H, *J* = 7.6, *J́* = 2.0 Hz, H_{pyr}-4), 8.55 (ddd, 1H, *J* = 4.8, *J́* = 2.0, *J́* = 0.8 Hz, H_{pyr}-6). IR *ν* (cm^{-1}) 3328, 1733, 1608, 1593, 1572, 1499. MS (ESI) *m*/*z* 419 $[M+H]^+$. Anal. Calcd for $C_{27}H_{34}N_2O_2$: C, 77.48; H, 8.19; N, 6.69. Found: C, 77.40; H, 8.25; N, 6.66%.

4.17. 3-(3-(2-(Pyridin-2-yl)ethylamino)propoxy)estra-1,3,5(10)-trien-17-one (13b)

Compound **13b** was prepared as a pale yellow oil from **5** according to the general procedure B using 2-(2-aminoethyl)pyridine. Yield: 79%, $[\alpha]_D$ +87° (*c* 0.38). ^{1}H NMR ($CDCl_3$) δ 0.91 (s, 3H, CH_3), 2.25 (m, 1H, H-9), 2.40 (m, 1H, H-11α), 2.50 (dd, 1H, *J* = 19.0, *J́* = 8.5 Hz, H-16β), 2.84 (t, 2H, *J* = 6.8 Hz, NHCH_2), 2.84–2.91 (m, 2H, H-6α, H-6β), 2.97–3.07 (m, 4H, PyrCH_2CH_2), 4.00 (t, 2H, *J* = 6.0 Hz, CH_2O), 6.62 (d, 1H, *J* = 2.8 Hz, H-4), 6.68 (dd, 1H, *J* = 8.6, *J́* = 2.8 Hz, H-2), 7.24 (ddd, 1H, *J* = 7.6, *J́* = 4.8, *J́* = 1.1 Hz, H_{pyr}-5), 7.16–7.19 (m, 2H, H-1, H_{pyr}-3), 7.58 (td, 1H, *J* = 7.6, *J́* = 1.6 Hz, H_{pyr}-4), 8.51 (ddd, 1H, *J* = 4.8, *J́* = 1.6, *J́* = 0.8 Hz, H_{pyr}-6). IR *ν* (cm^{-1}) 3324, 1733, 1608, 1594, 1571, 1499. MS (ESI) *m*/*z* 433 $[M+H]^+$. Anal. Calcd for $C_{28}H_{36}N_2O_2$: C, 77.74; H, 8.39; N, 6.48. Found: C, 77.69; H, 8.40; N, 6.45%.

4.18. 3-(3-(Pyridin-2-ylmethylamino)propoxy)estra-1,3,5(10)-trien-17β-ol (14a)

Compound **14a** was prepared as a pale yellow oil from **6** according to the general procedure B using 2-(aminomethyl)pyridine. Yield: 85%, $[\alpha]_D$ +46° (*c* 0.26). ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H, CH_3), 1.70 (m, 1H, H-15α), 1.87 (m, 1H, H-12β), 1.94 (td, 1H, *J* = 12.2, *J́* = 3.6 Hz, H-7β), 2.01 (t, 2H, *J* = 6.8 Hz, $NHCH_2CH_2$), 2.09–2.21 (m, 2H, H-9, H-16α), 2.31 (m, 1H, H-11α), 2.78–2.89 (m, 4H, H-6α, H-6β, NHCH_2CH_2), 3.73 (t, 1H, *J* = 8.4 Hz, H-17α), 3.93 (s, 2H, PyrCH_2NH), 4.03 (t, 2H, *J* = 6.3 Hz, CH_2O), 6.63 (d, 1H, *J* = 2.4 Hz, H-4), 6.70 (dd, 1H, *J* = 8.4, *J́* = 2.4 Hz, H-2), 7.16 (ddd, 1H, *J* = 7.5, *J́* = 4.9, *J́* = 1.2 Hz, H_{pyr}-5), 7.19 (d, 1H, *J* = 8.4 Hz, H-1), 7.30 (br d, 1H, *J* = 7.5 Hz, H_{pyr}-3), 7.64 (td, 1H, *J* = 7.5, *J́* = 1.6 Hz, H_{pyr}-4), 8.55 (ddd, 1H, *J* = 4.9, *J́* = 1.6, *J́* = 0.8 Hz, H_{pyr}-6). IR *ν* (cm^{-1}) 3613, 3325, 1607, 1594, 1572, 1499. MS (ESI) *m*/*z* 421 $[M+H]^+$. Anal. Calcd for $C_{27}H_{36}N_2O_2$: C, 77.10; H, 8.63; N, 6.66. Found: C, 77.05; H, 8.67; N, 6.60%.

4.19. 3-(3-(2-(Pyridin-2-yl)ethylamino)propoxy)estra-1,3,5(10)-trien-17β-ol (14b)

Compound **14b** was prepared as a pale yellow oil from **6** according to the general procedure B using 2-(2-aminoethyl)pyridine. Yield: 82%, $[\alpha]_D$ +48° (*c* 0.23). ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3H, CH_3), 1.69 (m, 1H, H-15α), 1.87 (m, 1H, H-12β), 1.93 (m, 1H, H-7β), 2.07–2.21 (m, 2H, H-9, H-16α), 2.30 (m, 1H, H-11α), 2.79–2.88 (m, 4H, H-6α, H-6β, NHCH_2CH_2), 2.98–3.07 (m, 4H, PyrCH_2CH_2), 3.73 (t, 1H, *J* = 8.4 Hz, H-17α), 3.99 (t, 2H, *J* = 6.4 Hz, CH_2O), 6.60 (d, 1H, *J* = 2.4 Hz, H-4), 6.67 (dd, 1H, *J* = 8.4, *J́* = 2.4 Hz, H-2), 7.11 (ddd, 1H, *J* = 7.6, *J́* = 5.0, *J́* = 1.2 Hz, H_{pyr}-5), 7.16–7.19 (m, 2H, H-1, H_{pyr}-3), 7.58 (td, 1H, *J* = 7.6, *J́* = 1.9 Hz, H_{pyr}-4), 8.50 (ddd, 1H, *J* = 5.0, *J́* = 1.6, *J́* = 0.8 Hz, H_{pyr}-6). IR *ν* (cm^{-1}) 3613, 3323, 1607, 1594, 1571, 1499. MS (ESI) *m*/*z* 435 $[M+H]^+$. Anal. Calcd for $C_{28}H_{38}N_2O_2$: C, 77.38; H, 8.81; N, 6.45. Found: C, 77.33; H, 8.86; N, 6.44%.

4.20. 3-(4-(Pyridin-2-ylmethylamino)butoxy)estra-1,3,5(10)-trien-17-one (15a)

Compound **15a** was prepared as a pale yellow powder from **7** according to the general procedure B using 2-(aminomethyl)pyridine. Yield: 83%, mp 86–88 °C, $[\alpha]_D$ +99° (*c* 0.20). ^{1}H NMR ($CDCl_3$) δ 0.91 (s, 3H, CH_3), 2.25 (m, 1H, H-9), 2.39 (m, 1H, H-11α), 2.50 (dd, 1H, *J* = 19.0, *J́* = 8.8 Hz, H-16β), 2.74 (t, 2H, *J* = 7.2 Hz, NHCH_2CH_2), 2.81–2.93 (m, 2H, H-6α, H-6β,), 3.93 (s, 2H, PyrCH_2NH), 3.95 (t, 2H, *J* = 6.3 Hz, CH_2O), 6.63 (d, 1H, *J* = 2.8 Hz, H-4), 6.70 (dd, 1H, *J* = 8.5, *J́* = 2.8 Hz, H-2), 7.15–7.20 (m, 2H, H-1, H_{pyr}-5), 7.31 (br d, 1H, *J* = 7.6 Hz, H_{pyr}-3), 7.64 (td, 1H, *J* = 7.9, *J́* = 1.6 Hz, H_{pyr}-4), 8.56 (ddd, 1H, *J* = 4.8, *J́* = 1.6, *J́* = 0.8 Hz, H_{pyr}-6). IR *ν* (cm^{-1}) 3318, 1733, 1608, 1593, 1572, 1499. MS (ESI) *m*/*z* 433 $[M+H]^+$. Anal. Calcd for $C_{28}H_{36}N_2O_2$: C, 77.74; H, 8.39; N, 6.48. Found: C, 77.71; H, 8.41; N, 6.48%.

4.21. 3-(4-(2-(Pyridin-2-yl)ethylamino)butoxy)estra-1,3,5(10)-trien-17-one (15b)

Compound **15b** was prepared as a pale yellow powder from **7** according to the general procedure B using 2-(2-aminoethyl)pyridine. Yield: 82%, mp 85–87 °C, $[\alpha]_D$ +100° (*c* 0.24). ^{1}H NMR ($CDCl_3$) δ 0.91 (s, 3H, CH_3), 2.25 (m, 1H, H-9), 2.39 (m, 1H, H-11α), 2.50 (dd, 1H, *J* = 18.8, *J́* = 8.4 Hz, H-16β), 2.73 (t, 2H, *J* = 7.1 Hz, NHCH_2), 2.84–2.91 (m, 2H, H-6α, H-6β), 2.98–3.07 (m, 4H, PyrCH_2CH_2), 3.94 (t, 2H, *J* = 6.3 Hz, CH_2O), 6.63 (d, 1H, *J* = 2.8 Hz, H-4), 6.69 (dd, 1H, *J* = 8.4, *J́* = 2.8 Hz, H-2), 7.13 (ddd, 1H, *J* = 7.6, *J́* = 4.8, *J́* = 1.2 Hz, H_{pyr}-5), 7.16-7.19 (m, 2H, H-1, H_{pyr}-3), 7.60 (td, 1H, *J* = 7.6, *J́* = 1.6 Hz, H_{pyr}-4), 8.53 (ddd, 1H, *J* = 4.8, *J́* = 1.6, *J́* = 0.8 Hz, H_{pyr}-6). IR *ν* (cm^{-1}) 3306, 1733, 1608, 1594, 1571, 1499. MS (ESI) *m*/*z* 447 $[M+H]^+$. Anal. Calcd for $C_{29}H_{38}N_2O_2$: C, 77.99; H, 8.58; N, 6.27. Found: C, 77.95; H, 8.67; N, 6.29%.

4.22. 3-(4-(Pyridin-2-ylmethylamino)butoxy)estra-1,3,5(10)-trien-17β-ol (16a)

Compound **16a** was prepared as a pale yellow oil from **8** according to the general procedure B using 2-(aminomethyl)

pyridine. Yield: 84%, $[\alpha]_D$ +40° (*c* 0.27). ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H, CH_3), 1.94 (td, 1H, *J* = 12.3, *J́* = 2.8 Hz, H-7β), 2.07–2.21 (m, 2H, H-9, H-16α), 2.31 (m, 1H, H-11α), 2.74 (t, 2H, *J* = 6.8 Hz, NHC***H*****$_2$**CH_2), 2.78–2.90 (m, 2H, H-6α, H-6β), 3.73 (t, 1H, *J* = 8.4 Hz, H-17α), 3.93 (s, 2H, PyrC***H*****$_2$**NH), 3.94 (t, 2H, *J* = 6.4 Hz, CH_2O), 6.61 (d, 1H, *J* = 2.4 Hz, H-4), 6.69 (dd, 1H, *J* = 8.6, *J́* = 2.4 Hz, H-2), 7.15–7.20 (m, 2H, H-1, H_{pyr}-5), 7.31 (br d, 1H, *J* = 7.6 Hz, H_{pyr}-3), 7.64 (td, 1H, *J* = 7.6, *J́* = 1.6 Hz, H_{pyr}-4), 8.55 (ddd, 1H, *J* = 4.8, *J́* = 1.6, *J́* = 0.8 Hz, H_{pyr}-6). IR *ν* (cm^{-1}) 3615, 3318, 1607, 1594, 1572, 1499. MS (ESI) *m*/*z* 435 $[M+H]^+$. Anal. Calcd for $C_{28}H_{38}N_2O_2$: C, 77.38; H, 8.81; N, 6.45. Found: C, 77.35; H, 8.82; N, 6.41%.

4.23. 3-(4-(2-(Pyridin-2-yl)ethylamino)butoxy)estra-1,3,5(10)-trien-17β-ol (16b)

Compound **16b** was prepared as a pale yellow oil from **8** according to the general procedure B using 2-(2-aminoethyl)pyridine. Yield: 84%, $[\alpha]_D$ +54° (*c* 0.26). ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3H, CH_3), 1.94 (td, 1H, *J* = 12.3, *J́* = 2.8 Hz, H-7β), 2.07–2.21 (m, 2H, H-9, H-16α), 2.30 (m, 1H, H-11α), 2.71 (t, 2H, *J* = 7.0 Hz, NHC***H*****$_2$**CH_2), 2.79–2.88 (m, 2H, H-6α, H-6β), 2.97–3.06 (m, 4H, PyrC***H*****$_2$C*H*$_2$**), 3.73 (t, 1H, *J* = 8.4 Hz, H-17α), 3.93 (t, 2H, *J* = 6.1 Hz, CH_2O), 6.60 (d, 1H, *J* = 2.6 Hz, H-4), 6.68 (dd, 1H, *J* = 8.4, *J́* = 2.6 Hz, H-2), 7.12 (ddd, 1H, *J* = 7.4, *J́* = 4.9, *J́* = 1.2 Hz, H_{pyr}-5), 7.16-7.19 (m, 2H, H-1, H_{pyr}-3), 7.59 (td, 1H, *J* = 7.6, *J́* = 1.6 Hz, H_{pyr}-4), 8.53 (ddd, 1H, *J* = 4.9, *J́* = 1.6, *J́* = 0.8 Hz, H_{pyr}-6). IR *ν* (cm^{-1}) 3614, 3311, 1607, 1594, 1571, 1499. MS (ESI) *m*/*z* 449 $[M+H]^+$. Anal. Calcd for $C_{29}H_{40}N_2O_2$: C, 77.64; H, 8.99; N, 6.24. Found: C, 77.59; H, 9.07; N, 6.20%.

4.24. 3-(5-(Pyridin-2-ylmethylamino)pentoxy)estra-1,3,5(10)-trien-17-one (17a)

Compound **17a** was prepared as a pale yellow oil from **9** according to the general procedure B using 2-(aminomethyl)pyridine. Yield: 85%, $[\alpha]_D$ +84° (*c* 0.21). ^{1}H NMR ($CDCl_3$) δ 0.91 (s, 3H, CH_3), 2.25 (m, 1H, H-9), 2.39 (m, 1H, H-11α), 2.50 (dd, 1H, *J* = 19.1, *J́* = 8.8 Hz, H-16β), 2.69 (t, 2H, *J* = 7.2 Hz, NHC***H*****$_2$**CH_2), 2.82–2.95 (m, 2H, H-6α, H-6β), 3.92 (s, 2H, PyrC***H*****$_2$**NH), 3.93 (t, 2H, *J* = 6.7 Hz, CH_2O), 6.63 (d, 1H, *J* = 2.6 Hz, H-4), 6.70 (dd, 1H, *J* = 8.6, *J́* = 2.6 Hz, H-2), 7.15–7.20 (m, 2H, H-1, H_{pyr}-5), 7.30 (br d, 1H, *J* = 7.8 Hz, H_{pyr}-3), 7.64 (td, 1H, *J* = 7.7, *J́* = 1.8 Hz, H_{pyr}-4), 8.57 (ddd, 1H, *J* = 4.8, *J́* = 1.5, *J́* = 0.7 Hz, H_{pyr}-6). IR *ν* (cm^{-1}) 3318, 1733, 1608, 1593, 1572, 1499. MS (ESI) *m*/*z* 447 $[M+H]^+$. Anal. Calcd for $C_{29}H_{38}N_2O_2$: C, 77.99; H, 8.58; N, 6.27. Found: C, 80.01; H, 8.63; N, 6.20%.

4.25. 3-(5-(2-(Pyridin-2-yl)ethylamino)pentoxy)estra-1,3,5(10)-trien-17-one (17b)

Compound **17b** was prepared as a pale yellow oil from **9** according to the general procedure B using 2-(2-aminoethyl)pyridine. Yield: 84%, $[\alpha]_D$ +76° (*c* 0.25). ^{1}H NMR ($CDCl_3$) δ 0.91 (s, 3H, CH_3), 2.25 (m, 1H, H-9), 2.40 (m, 1H, H-11α), 2.50 (dd, 1H, *J* = 18.7, *J́* = 8.5 Hz, H-16β), 2.68 (t, 2H, *J* = 6.9 Hz, NHC***H*****$_2$**), 2.87–2.91 (m, 2H, H-6α, H-6β), 2.97–3.06 (m, 4H, PyrC***H*****$_2$C*H*$_2$**), 3.92 (t, 2H, *J* = 6.9 Hz, CH_2O), 6.63 (d, 1H, *J* = 2.6 Hz, H-4), 6.70 (dd, 1H, *J* = 8.6, *J́* = 2.6 Hz, H-2), 7.12 (dd, 1H, *J* = 7.5, *J́* = 4.9 Hz, H_{pyr}-5), 7.17–7.20 (m, 2H, H-1, H_{pyr}-3), 7.60 (td, 1H, *J* = 7.7, *J́* = 1.8 Hz, H_{pyr}-4), 8.53 (ddd, 1H, *J* = 4.8, *J́* = 1.5, *J́* = 0.7 Hz, H_{pyr}-6). IR *ν* (cm^{-1}) 3307, 1733, 1608, 1594, 1571, 1499. MS (ESI) *m*/*z* 461 $[M+H]^+$. Anal. Calcd for $C_{30}H_{40}N_2O_2$: C, 78.22; H, 8.75; N, 6.08. Found: C, 78.16; H, 8.81; N, 6.04%.

4.26. 3-(5-(Pyridin-2-ylmethylamino)pentoxy)estra-1,3,5(10)-trien-17β-ol (18a)

Compound **18a** was prepared as a pale yellow powder from **10** according to the general procedure B using 2-(aminomethyl)pyridine. Yield: 82%, mp 112–114 °C, $[\alpha]_D$ +61° (*c* 0.24). ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H, CH_3), 1.94 (td, 1H, *J* = 12.5, *J́* = 3.4 Hz, H-7β), 2.04–2.24 (m, 2H, H-9, H-16α), 2.31 (ddd, 1H, *J* = 13.2, *J́* = 6.9, *J́* = 3.9 Hz, H-11α), 2.69 (t, 2H, *J* = 7.0 Hz, NHC***H*****$_2$**CH_2), 2.75–2.94 (m, 2H, H-6α, H-6β), 3.73 (t, 1H, *J* = 8.5 Hz, H-17α), 3.91 (s, 2H, PyrC***H*****$_2$**NH), 3.92 (t, 2H, *J* = 6.5 Hz, CH_2O), 6.61 (d, 1H, *J* = 2.6 Hz, H-4), 6.69 (dd, 1H, *J* = 8.6, *J́* = 2.6 Hz, H-2), 7.11–7.20 (m, 2H, H-1, H_{pyr}-5), 7.30 (br d, 1H, *J* = 7.8 Hz, H_{pyr}-3), 7.64 (td, 1H, *J* = 7.7, *J́* = 1.8 Hz, H_{pyr}-4), 8.55 (ddd, 1H, *J* = 4.9, *J́* = 1.7, *J́* = 0.9 Hz, H_{pyr}-6). IR *ν* (cm^{-1}) 3613, 3316, 1607, 1594, 1572, 1499. MS (ESI) *m*/*z* 449 $[M+H]^+$. Anal. Calcd for $C_{29}H_{40}N_2O_2$: C, 77.64; H, 8.99; N, 6.24. Found: C, 77.58; H, 9.06; N, 6.19%.

4.27. 3-(5-(2-(Pyridin-2-yl)ethylamino)pentoxy)estra-1,3,5(10)-trien-17β-ol (18b)

Compound **18b** was prepared as a pale yellow oil from **10** according to the general procedure B using 2-(2-aminoethyl)pyridine. Yield: 83%, $[\alpha]_D$ +49° (*c* 0.16). ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3H, CH_3), 1.94 (td, 1H, *J* = 12.4, *J́* = 3.2 Hz, H-7β), 2.07–2.21 (m, 2H, H-9, H-16α), 2.31 (m, 1H, H-11α), 2.67 (t, 2H, *J* = 7.1 Hz, NHC***H*****$_2$**CH_2), 2.76–2.90 (m, 2H, H-6α, H-6β), 2.94–3.08 (m, 4H, PyrC***H*****$_2$C*H*$_2$**), 3.73 (t, 1H, *J* = 8.5 Hz, H-17α), 3.91 (t, 2H, *J* = 6.5 Hz, CH_2O), 6.61 (d, 1H, *J* = 2.6 Hz, H-4), 6.69 (dd, 1H, *J* = 8.6, *J́* = 2.6 Hz, H-2), 7.12 (ddd, 1H, *J* = *J* = 7.5, *J́* = 4.9, *J́* = 0.9 Hz, H_{pyr}-5), 7.16–7.20 (m, 2H, H-1, H_{pyr}-3), 7.60 (td, 1H, *J* = 7.7, *J́* = 1.8 Hz, H_{pyr}-4), 8.53 (ddd, 1H, *J* = 4.9, *J́* = 1.6, *J́* = 0.8 Hz, H_{pyr}-6). IR *ν* (cm^{-1}) 3613, 3306, 1607, 1594, 1571, 1499. MS (ESI) *m*/*z* 463 $[M+H]^+$. Anal. Calcd for $C_{30}H_{42}N_2O_2$: C, 77.88; H, 9.15; N, 6.05. Found: C, 77.80; H, 9.22; N, 5.99%.

4.28. General procedure for the preparation of the platinum complexes (C)

The solution of potassium tetrachloroplatinate (90 mg, 0.22 mmol) in DMF (2 mL) and distilled water (2 mL) were added to a solution of steroidal ligand (0.22 mmol) in DMF (2 mL). The resulting mixture was stirred in the dark for 3 days. Then, the solvent was evaporated and the residue was stirred vigorously in a saturated aqueous potassium chloride solution (5 mL) for 20 min. The resulting suspension was filtered, washed with water, and dried in a desiccator over phosphorus pentoxide for 1 day. All complexes were prepared as precipitated off-white powder and due to sufficient purity (according to elemental analysis) were not recrystallized.

4.29. *cis*-Dichloro[3-(2-(pyridin-2-ylmethylamino-κN1,κN2)ethoxy)estra-1,3,5(10)-trien-17-one]platinum(II) (19a)

Compound **19a** was prepared from **11a** according to the general procedure C. Yield: 91%, mp 264–267 °C (decomp.), $[\alpha]_D$ +85° (*c* 0.31, DMF). IR (KBr) *ν* (cm^{-1}) 3143, 3113, 1739, 1612, 1593, 1573, 1499. MS (ESI) *m*/*z* 670, 671, 672 $[M+H]^+$, 691, 692, 693, 694, 695 $[M+Na]^+$. Anal. Calcd for $C_{26}H_{32}Cl_2N_2O_2Pt{\cdot}H_2O$: C, 45.35; H, 4.98; N, 4.07. Found: C, 45.29; H, 4.80; N, 4.05%.

4.30. *cis*-Dichloro[3-(2-(2-(pyridin-2-yl-κN)ethylamino-κN)ethoxy)estra-1,3,5(10)-trien-17-one]platinum(II) (19b)

Compound **19b** was prepared from **11b** according to the general procedure C. Yield: 89%, mp 268–272 °C (decomp.), $[\alpha]_D$ +65° (*c*

0.11). ^{1}H NMR ($CDCl_3$) δ 0.91 (s, 3H, CH_3), 1.94–2.22 (m, 5H), 2.36 (m, 1H, H-11α), 2.51 (dd, 1H, J = 18.8, J' = 8.6 Hz, H-16β), 2.66 (dd, 1H, J = 20.6, J' = 10.6 Hz), 2.73–2.93 (m, 2H), 3.02 (dd, 1H, J = 15.3, J' = 3.0 Hz), 3.23 (m, 1H), 3.36 (m, 2H), 4.17 (m, 1H), 4.31 (t, 1H, J = 13.2 Hz), 4.90 (m, 1H), 6.37–6.43 (m, 2H), 6.65 (br s, 1H, NH), 6.99 (m, 1H), 7.24 (ddd, 1H, J = 7.6, J' = 4.9, J' = 1.1 Hz), 7.31 (br d, 1H, J = 7.7 Hz), 7.70 (tdd, 1H, J = 7.7, J' = 4.2, J' = 1.5 Hz), 9.01 (ddd, 1H, J = 11.0, J' = 6.0, J' = 1.0 Hz). IR (KBr) ν (cm^{-1}) 3166, 3127, 1739, 1610, 1574, 1538, 1498. MS (ESI) m/z 705, 706, 707, 708, 709 $[M+Na]^+$. Anal. Calcd for $C_{27}H_{34}Cl_2N_2O_2Pt{\cdot}H_2O$: C, 46.16; H, 5.16; N, 3.99. Found: C, 46.05; H, 5.01; N, 3.81%.

4.31 *cis*-Dichloro[3-(2-(pyridin-2-ylmethylamino-κN1,κN2)ethoxy)estra-1,3,5(10)-trien-17β-ol]platinum(II) (20a)

Compound **20a** was prepared from **12a** according to the general procedure C. Yield: 88%, mp 275–280 °C (decomp.), $[\alpha]_D$ +55° (c 0.17, DMF). IR (KBr) ν (cm^{-1}) 3613, 3143, 3114, 1608, 1574, 1498. MS (ESI) m/z 693, 694, 695, 696, 697 $[M+Na]^+$. Anal. Calcd for $C_{26}H_{34}Cl_2N_2O_2Pt{\cdot}H_2O$: C, 45.22; H, 5.25; N, 4.06. Found: C, 45.28; H, 5.28; N, 4.00%.

4.32. *cis*-Dichloro[3-(2-(2-(pyridin-2-yl-κN)ethylamino-κN)ethoxy)estra-1,3,5(10)-trien-17β-ol]platinum(II) (20b)

Compound **20b** was prepared from **12b** according to the general procedure C. Yield: 88%, mp 179–182 °C, $[\alpha]_D$ +43° (c 0.13). ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3H, CH_3), 1.87 (m, 1H), 1.95 (dt, 1H, J = 12.2, J' = 2.8 Hz), 2.10–2.17 (m, 2H), 2.28 (m, 1H, H-11α), 2.62–2.84 (m, 3H), 3.01 (d, 1H, J = 14.3 Hz), 3.16–3.45 (m, 3H), 3.73 (t, 1H, J = 8.4 Hz, H-17α), 4.16 (m, 1H), 4.33 (m, 1H), 4.95 (m, 1H), 6.32 (dd, 1H, J = 10.6, J' = 2.6 Hz), 6.40 (td, 1H, J = 9.1, J' = 2.7 Hz), 6.74 (br s, 1H, NH), 6.95 (m, 1H), 7.06 (dd, 1H, J = 8.6, J' = 1.6 Hz), 7.20 (d, 1H, J = 7.8 Hz), 7.68 (m, 1H), 8.98 (ddd, 1H, J = 11.0, J' = 6.0, J' = 1.0 Hz). IR ν (cm^{-1}) 3613, 3241, 3144, 1610, 1573, 1488. MS (ESI) m/z 707, 708, 709, 710, 711 $[M+Na]^+$. Anal. Calcd for $C_{27}H_{36}Cl_2N_2O_2Pt{\cdot}½H_2O$: C, 46.62; H, 5.36; N, 4.03. Found: C, 46.55; H, 5.42; N, 3.89%.

4.33. *cis*-Dichloro[3-(3-(pyridin-2-ylmethylamino-κN1,κN2)propoxy)estra-1,3,5(10)-trien-17-one]platinum(II) (21a)

Compound **21a** was prepared from **13a** according to the general procedure C. Yield: 86%, mp 170–172 °C, $[\alpha]_D$ +56° (c 0.25, DMF). IR (KBr) ν (cm^{-1}) 3140, 3120, 1735, 1610, 1573, 1498. MS (ESI) m/z 684, 685, 686 $[M+H]^+$, 705, 706, 707, 708, 709 $[M+Na]^+$. Anal. Calcd for $C_{27}H_{34}Cl_2N_2O_2Pt{\cdot}H_2O$: C, 46.16; H, 5.16; N, 3.99. Found: C, 46.03; H, 5.29; N, 4.00%.

4.34. *cis*-Dichloro[3-(3-(2-(pyridin-2-yl-κN)ethylamino-κN)propoxy)estra-1,3,5(10)-trien-17-one]platinum(II) (21b)

Compound **21b** was prepared from **13b** according to the general procedure C. Yield: 91%, mp 154–156 °C, $[\alpha]_D$ +51° (c 0.17). ^{1}H NMR ($CDCl_3$) δ 0.90 (s, 3H, CH_3), 1.90–2.25 (m, 7H), 2.29–2.41 (m, 2H), 2.50 (dd, 1H, J = 18.8, J' = 8.6 Hz, H-16β), 2.85–2.89 (m, 2H), 2.98–3.16 (m, 2H), 3.24–3.38 (m, 2H), 3.95–4.04 (m, 2H), 4.37 (t, 1H, J = 12.7 Hz), 6.46 (m, 1H, NH), 6.58 (d, 1H, J = 2.5 Hz), 6.64 (dd, 1H, J = 8.6, J' = 2.7 Hz), 7.16 (d, 1H, J = 8.6 Hz), 7.26–7.29 (m, 2H), 7.83 (td, 1H, J = 7.7, J' = 1.5 Hz), 9.24 (m, 1H). IR ν (cm^{-1}) 3239, 3145, 1733, 1611, 1573, 1499, 1485. MS (ESI) m/z 719, 720, 721, 722, 723 $[M+Na]^+$. Anal. Calcd for $C_{28}H_{36}Cl_2N_2O_2Pt{\cdot}H_2O$: C, 46.93; H, 5.34; N, 3.91. Found: C, 46.77; H, 5.39; N, 3.99%.

4.35. *cis*-Dichloro[3-(3-(pyridin-2-ylmethylamino-κN1,κN2)propoxy)estra-1,3,5(10)-trien-17β-ol]platinum(II) (22a)

Compound **22a** was prepared from **14a** according to the general procedure C. Yield: 86%, mp 166–168 °C, $[\alpha]_D$ +52° (c 0.14, DMF). IR (KBr) ν (cm^{-1}) 3481, 3324, 3116, 1609, 1573, 1498. MS (ESI) m/z 707, 708, 709, 710, 711 $[M+Na]^+$. Anal. Calcd for $C_{27}H_{36}Cl_2N_2O_2Pt{\cdot}H_2O$: C, 46.03; H, 5.44; N, 3.98. Found: C, 46.07; H, 5.30; N, 3.82%.

4.36. *cis*-Dichloro[3-(3-(2-(pyridin-2-yl-κN)ethylamino-κN)propoxy)estra-1,3,5(10)-trien-17β-ol]platinum(II) (22b)

Compound **22b** was prepared from **14b** according to the general procedure C. Yield: 87%, mp 150–152 °C, $[\alpha]_D$ +61° (c 0.20). ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H, CH_3), 1.85 (m, 1H), 1.94 (dt, 1H, J = 12.4, J' = 3.0 Hz), 1.99–2.21 (m, 4H), 2.26–2.39 (m, 2H), 2.77–2.84 (m, 2H), 3.04–3.11 (m, 2H), 3.26–3.36 (m, 2H), 3.72 (t, 1H, J = 8.5 Hz, H-17α), 3.94–4.03 (m, 2H), 4.34 (m, 1H), 6.42 (m, 1H, NH), 6.56 (d, 1H, J = 2.6 Hz), 6.62 (dd, 1H, J = 8.6, J' = 2.6 Hz), 7.16 (d, 1H, J = 8.6 Hz), 7.25–7.29 (m, 2H), 7.82 (td, 1H, J = 7.7, J' = 1.5 Hz), 9.23 (m, 1H). IR ν (cm^{-1}) 3612, 3239, 3146, 1610, 1572, 1499, 1485. MS (ESI) m/z 721, 722, 723, 724, 725 $[M+Na]^+$. Anal. Calcd for $C_{28}H_{38}Cl_2N_2O_2Pt{\cdot}H_2O$: C, 46.80; H, 5.61; N, 3.90. Found: C, 46.75; H, 5.60; N, 3.77%.

4.37. *cis*-Dichloro[3-(4-(pyridin-2-ylmethylamino-κN1,κN2)butoxy)estra-1,3,5(10)-trien-17-one]platinum(II) (23a)

Compound **23a** was prepared from **15a** according to the general procedure C. Yield: 90%, mp 165–167 °C, $[\alpha]_D$ +64° (c 0.15, DMF). ^{1}H NMR ($CDCl_3$) δ 0.90 (s, 3H, CH_3), 1.79–1.89 (m, 3H), 1.91–2.27 (m, 6H), 2.37 (m, 1H, H-11α), 2.50 (dd, 1H, J = 18.8, J' = 8.5 Hz, H-16β), 2.83–2.91 (m, 2H), 3.07 (m, 1H), 3.19 (m, 1H), 3.93 (t, 2H, J = 5.5 Hz), 4.00 (d, 1H, J = 16.7 Hz), 5.31 (dt, 1H, J = 11.8, J' = 7.3 Hz), 6.49 (m, 1H, NH), 6.61 (d, 1H, J = 2.6 Hz), 6.66 (dd, 1H, J = 8.6, J' = 2.6 Hz), 7.16–7.21 (m, 2H), 7.41 (d, 1H, J = 7.8 Hz), 7.90 (td, 1H, J = 7.8, J' = 1.4 Hz), 9.02 (m, 1H). IR (KBr) ν (cm^{-1}) 3117, 1735, 1611, 1572, 1498. MS (ESI) m/z 698, 699, 700 $[M+H]^+$, 719, 720, 721, 722, 723 $[M+Na]^+$. Anal. Calcd for $C_{28}H_{36}Cl_2N_2O_2Pt$: C, 48.14; H, 5.19; N, 4.01. Found: C, 48.27; H, 5.30; N, 3.95%.

4.38. *cis*-Dichloro[3-(4-(2-(pyridin-2-yl-κN)ethylamino-κN)butoxy)estra-1,3,5(10)-trien-17-one]platinum(II) (23b)

Compound **23b** was prepared from **15b** according to the general procedure C. Yield: 87%, mp 144–146 °C, $[\alpha]_D$ +52° (c 0.28). ^{1}H NMR ($CDCl_3$) δ 0.90 (s, 3H, CH_3), 1.80 (pentet, 2H, J = 6.5 Hz), 1.93–2.19 (m, 6H), 2.24 (m, 1H), 2.38 (m, 1H, H-11α), 2.50 (dd, 1H, J = 18.8, J' = 8.5 Hz, H-16β), 2.86–2.98 (m, 2H), 3.04 (dd, 1H, J = 15.4, J' = 2.6 Hz), 3.15–3.29 (m, 2H), 3.91 (t, 2H, J = 6.0 Hz), 4.45 (m, 1H), 6.46 (m, 1H, NH), 6.59 (d, 1H, J = 2.4 Hz), 6.64 (dd, 1H, J = 8.5, J' = 2.6 Hz), 7.17 (d, 1H, J = 8.6 Hz), 7.23–7.27 (m, 2H), 7.81 (td, 1H, J = 7.7, J' = 1.4 Hz), 9.23 (m, 1H). IR ν (cm^{-1}) 3240, 3147, 1733, 1611, 1572, 1499, 1485. MS (ESI) m/z 733, 734, 735, 736, 737 $[M+Na]^+$. Anal. Calcd for $C_{29}H_{38}Cl_2N_2O_2Pt{\cdot}H_2O$: C, 47.67; H, 5.52; N, 3.83. Found: C, 47.77; H, 5.48; N, 3.68%.

4.39. *cis*-Dichloro[3-(4-(pyridin-2-ylmethylamino-κN1,κN2) butoxy)estra-1,3,5(10)-trien-17β-ol]platinum(II) (24a)

Compound **24a** was prepared from **16a** according to the general procedure C. Yield: 88%, mp 185–190 °C (decomp.), $[\alpha]_D$ +21° (c 0.21, DMF). IR (KBr) ν (cm^{-1}) 3465, 3158, 1609, 1574, 1499. MS

(ESI) *m*/*z* 721, 722, 723, 724, 725 $[M+Na]^+$. Anal. Calcd for $C_{28}H_{38}Cl_2N_2O_2Pt$: C, 48.00; H, 5.47; N, 4.00. Found: C, 47.89; H, 5.58; N, 3.88%.

4.40. *cis*-Dichloro[3-(4-(2-(pyridin-2-yl-κN)ethylamino-κN)butoxy)estra-1,3,5(10)-trien-17β-ol]platinum(II) (24b)

Compound **24b** was prepared from **16b** according to the general procedure C. Yield: 90%, mp 140–142 °C, $[\alpha]_D$ +26° (*c* 0.16). ^1H NMR ($CDCl_3$) δ 0.77 (s, 3H, CH_3), 1.80 (pentet, 2H, *J* = 6.5 Hz), 1.87 (m, 1H), 1.94 (dt, 1H, *J* = 12.2, *J́* = 3.0 Hz), 2.02–2.20 (m, 4H), 2.30 (m, 1H, H-11α), 2.77–2.86 (m, 2H), 3.03 (dd, 1H, *J* = 15.3, *J́* = 2.9 Hz), 3.13–3.29 (m, 2H), 3.73 (t, 1H, *J* = 8.4 Hz, H-17α), 3.90 (t, 2H, *J* = 6.0 Hz), 4.44 (m, 1H), 6.49 (m, 1H, NH), 6.56 (d, 1H, *J* = 2.6 Hz), 6.63 (dd, 1H, *J* = 8.6, *J́* = 2.6 Hz), 7.17 (d, 1H, *J* = 8.6 Hz), 7.23–7.26 (m, 2H), 7.80 (td, 1H, *J* = 7.7, *J́* = 1.5 Hz), 9.22 (dd, 1H, *J* = 6.5, *J́* = 1.5 Hz). IR *ν* (cm^{-1}) 3613, 3241, 3147, 1610, 1572, 1499, 1485. MS (ESI) *m*/*z* 735, 736, 737, 738, 739 $[M+Na]^+$. Anal. Calcd for $C_{29}H_{40}Cl_2N_2O_2Pt$: C, 48.74; H, 5.64; N, 3.92. Found: C, 48.59; H, 5.75; N, 3.81%.

4.41. *cis*-Dichloro[3-(5-(pyridin-2-ylmethylamino-κN1,κN2)pentoxy)estra-1,3,5(10)-trien-17-one]platinum(II) (25a)

Compound **25a** was prepared from **17a** according to the general procedure C. Yield: 91%, mp 169–171 °C, $[\alpha]_D$ +51° (*c* 0.13). ^1H NMR ($CDCl_3$) δ 0.90 (s, 3H, CH_3), 1.91–2.18 (m, 6H), 2.25 (m, 1H), 2.38 (m, 1H, H-11α), 2.50 (dd, 1H, *J* = 18.8, *J́* = 8.5 Hz, H-16β), 2.87–2.94 (m, 2H), 3.02 (m, 1H), 3.14 (m, 2H), 3.90 (t, 2H, *J* = 6.2 Hz), 3.98 (dd, 1H, *J* = 16.7, *J́* = 2.4 Hz), 5.35 (dd, 1H, *J* = 16.6, *J́* = 6.7 Hz), 6.44 (br s, 1H, NH), 6.62 (d, 1H, *J* = 2.6 Hz), 6.68 (dd, 1H, *J* = 8.5, *J́* = 2.6 Hz), 7.16-7.21 (m, 2H), 7.40 (d, 1H, *J* = 8.0 Hz), 7.90 (td, 1H, *J* = 7.8, *J́* = 1.4 Hz), 9.03 (dd, 1H, *J* = 5.9, *J́* = 0.7 Hz). IR *ν* (cm^{-1}) 3146, 1733, 1609, 1573, 1499. MS (ESI) *m*/*z* 712, 713, 714 $[M+H]^+$, 733, 734, 735, 736, 737 $[M+Na]^+$. Anal. Calcd for $C_{29}H_{38}Cl_2N_2O_2Pt$: C, 48.88; H, 5.37; N, 3.93. Found: C, 48.77; H, 5.46; N, 3.88%.

4.42. *cis*-Dichloro[3-(5-(2-(pyridin-2-yl-κN)ethylamino-κN)pentoxy)estra-1,3,5(10)-trien-17-one]platinum(II) (25b)

Compound **25b** was prepared from **17b** according to the general procedure C. Yield: 88%, mp 132–134 °C, $[\alpha]_D$ +53° (*c* 0.13). ^1H NMR ($CDCl_3$) δ 0.90 (s, 3H, CH_3), 1.89–2.18 (m, 6H), 2.24 (m, 1H), 2.38 (m, 1H, H-11α), 2.50 (dd, 1H, *J* = 18.8, *J́* = 8.5 Hz, H-16β), 2.86–2.94 (m, 2H), 3.03 (dd, 1H, *J* = 15.3, *J́* = 2.8 Hz), 3.15 (m, 1H), 3.25 (m, 1H), 3.89 (t, 2H, *J* = 6.1 Hz), 4.47 (m, 1H), 6.46 (m, 1H, NH), 6.60 (d, 1H, *J* = 2.5 Hz), 6.66 (dd, 1H, *J* = 8.5, *J́* = 2.5 Hz), 7.17 (d, 1H, *J* = 8.5 Hz), 7.23–7.27 (m, 2H), 7.80 (td, 1H, *J* = 7.7, *J́* = 1.5 Hz), 9.23 (d, 1H, *J* = 5.7 Hz). IR *ν* (cm^{-1}) 3242, 3148, 1733, 1611, 1572, 1499, 1485. MS (ESI) *m*/*z* 747, 748, 749, 750, 751 $[M+Na]^+$. Anal. Calcd for $C_{30}H_{40}Cl_2N_2O_2Pt{\cdot}2H_2O$: C, 47.24; H, 5.82; N, 3.67. Found: C, 47.37; H, 5.72; N, 3.50%.

4.43. *cis*-Dichloro[3-(5-(pyridin-2-ylmethylamino-κN1,κN2)pentoxy)estra-1,3,5(10)-trien-17β-ol]platinum(II) (26a)

Compound **26a** was prepared from **18a** according to the general procedure C. Yield: 88%, mp 208–212 °C (decomp.), $[\alpha]_D$ +28° (*c* 0.18, DMF). ^1H NMR (MeOD with addition of $CHCl_3$) δ 0.77 (s, 3H, CH_3), 1.88 (m, 1H), 1.96 (dt, 1H, *J* = 12.2, *J́* = 3.0 Hz), 1.98–2.10 (m, 2H), 2.18 (m, 1H), 2.30 (m, 1H, H-11α), 2.84 (m, 2H), 2.91 (m, 1H), 3.01 (m, 1H), 3.69 (t, 1H, *J* = 8.6 Hz, H-17α), 3.84 (br s, 1H, NH_2), 3.92 (t, 2H, *J* = 6.2 Hz), 4.01 (d, 1H, *J* = 16.4 Hz), 4.75 (d, 1H, *J* = 16.4 Hz), 6.60 (d, 1H, *J* = 2.8 Hz), 6.67 (dd, 1H, *J* = 8.6, *J́* = 2.8 Hz), 7.19 (d, 1H, *J* = 8.6 Hz), 7.34 (t, 1H, *J* = 6.6 Hz), 7.49 (d, 1H, *J* = 7.8 Hz), 8.00 (td, 1H, *J* = 7.8, *J́* = 1.6 Hz), 9.14 (dd, 1H, *J* = 5.9, *J́* = 1.6 Hz). IR (KBr) *ν* (cm^{-1}) 3506, 3153, 1612, 1572, 1498. MS (ESI) *m*/*z* 714, 715, 716 $[M+H]^+$, 735, 736, 737, 738, 739 $[M+Na]^+$. Anal. Calcd for $C_{29}H_{40}Cl_2N_2O_2Pt{\cdot}2H_2O$: C, 46.40; H, 5.91; N, 3.73. Found: C, 46.52; H, 5.83; N, 3.80%.

4.44. *cis*-Dichloro[3-(5-(2-(pyridin-2-yl-κN)ethylamino-κN)pentoxy)estra-1,3,5(10)-trien-17β-ol]platinum(II) (26b)

Compound **26b** was prepared from **18b** according to the general procedure C. Yield: 86%, mp 128–130 °C, $[\alpha]_D$ +20° (*c* 0.18). ^1H NMR ($CDCl_3$) δ 0.77 (s, 3H, CH_3), 1.83–1.97 (m, 3H), 2.02–2.20 (m, 3H), 2.30 (m, 1H, H-11α), 2.77–2.94 (m, 3H), 3.04 (dd, 1H, *J* = 15.2, *J́* = 3.0 Hz), 3.11 (m, 1H), 3.25 (m, 1H), 3.73 (t, 1H, *J* = 8.5 Hz, H-17α), 3.88 (t, 2H, *J* = 6.2 Hz), 4.48 (m, 1H), 6.52 (m, 1H, NH), 6.58 (d, 1H, *J* = 2.6 Hz), 6.65 (dd, 1H, *J* = 8.6, *J́* = 2.6 Hz), 7.17 (d, 1H, *J* = 8.6 Hz), 7.23–7.26 (m, 2H), 7.80 (td, 1H, *J* = 7.7, *J́* = 1.5 Hz), 9.22 (dd, 1H, *J* = 6.5, *J́* = 1.4 Hz). IR *ν* (cm^{-1}) 3613, 3242, 3148, 1610, 1572, 1499, 1485. MS (ESI) *m*/*z* 728, 729, 730 $[M+H]^+$, 749, 750, 751, 752, 753 $[M+Na]^+$. Anal. Calcd for $C_{30}H_{42}Cl_2N_2O_2Pt{\cdot}H_2O$: C, 48.26; H, 5.94; N, 3.75. Found: C, 48.12; H, 5.99; N, 3.71%.

4.45. Evaluation of the platinum(II) complexes stability in DMEM

The platinum complex **25a** (1 mg) was dissolved in 0.5 mL of dimethylformamide (DMF) followed by the addition of 1.5 mL of Dulbecco's Modified Eagle's Medium (DMEM; Sigma, MO, USA) supplemented with 10% of fetal calf serum, 2 mM of glutamine, 10,000 U of penicillin, and 10 mg/mL of streptomycin. This solution was incubated at 37 °C. After the given time (3, 4, 5, 24, 48, and 72 h), a sample of the solution was taken and the purity and concentration of the platinum complex was assessed by HPLC (mobile phase water/methanol; gradient elution: 20 min 100/0, 20–30 min 0/100; flow rate: 1 mL/min; detection: UV 300 nm). All of the fractions were analyzed by MS (ESI). The same procedure was followed for complex **25b**.

4.46. In vitro studies

4.46.1. Cell culture

The stock solutions (10 mM) of the platinum complexes were prepared by dissolving an appropriate quantity of each substance in DMF (Sigma, MO, USA). Dulbecco's modified Eagle's medium (DMEM), DMEM F12, RPMI 1640 medium, fetal bovine serum (FBS), L-glutamine, penicillin and streptomycin were purchased from Sigma (MO, USA). Calcein AM was obtained from Molecular Probes (Invitrogen Corporation, CA, USA).

The screening cell lines (T-lymphoblastic leukemia cell line CEM, human osteosarcoma cell line U-2 OS, estrogen dependent human breast adenocarcinoma cell line MCF7 (ER+), estrogen dependent human breast adenocarcinoma cell line—cultivated in steroid free medium MCF7 AL (ER+), estrogen independent human breast adenocarcinoma cell line MDA-MB-468 (ER−), an ERα (−) and ERβ (+) human breast ductal carcinoma cell line BT-474 (ER+), estrogen independent human breast ductal carcinoma BT-549 (ER−) and human fibroblasts BJ) were obtained from the American Type Culture Collection (Manassas, VA, USA). The CEM cells were cultured in RPMI 1640 medium (Sigma, MO, USA), supplemented with 15% of heat-inactivated fetal bovine serum, 2 mM of L-glutamine, 10,000 U of penicillin, and 10 mg/mL of streptomycin. The MCF7 AL cells were cultured in DMEM F12 medium

(Sigma, MO, USA), supplemented with 10% of heat-inactivated fetal bovine serum, 2 mM of L-glutamine, 10,000 U of penicillin, and 10 mg/mL of streptomycin. The MDA-MB-468 were cultured in DMEM medium (Sigma, MO, USA), supplemented with 10% of heat-inactivated fetal bovine serum, 2 mM of L-glutamine, 1 mM of sodium pyruvate, 15 mM of glucose, 10,000 U of penicillin and 10 mg/mL of streptomycin. All other cell lines were cultured in DMEM medium (Sigma, MO, USA), supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 10,000 U penicillin and 10 mg/mL streptomycin. The cell lines were maintained under standard cell culture conditions at 37 °C and 5% CO_2 in a humid environment. The cells were subcultured two or three times a week using the standard trypsinization procedure.

4.46.2. Cytotoxicity assay

The suspensions of the cell lines tested (ca. 1.0×10^5 cells/mL) were placed in 96-well microtiter plates and after 3 h of stabilization (time zero) the tested compounds were added (in four 20 μL aliquots) in serially diluted concentrations in dimethylformamide (DMF). The control cultures were treated with DMF alone, and the final concentration of DMF in the incubation mixtures never exceeded 0.6%. The compounds tested were typically evaluated at six 3-fold dilutions, with the highest final concentration being generally 50 μM (these conditions varied in a few cases, depending on the compound). After a 72 h incubation, 100 μl Calcein AM solution was added, and incubation continued for an additional hour. The fluorescence of the viable cells was then quantified using a Fluoroskan Ascent instrument (Labsystems, Finland). The percentage of surviving cells in each well was calculated by dividing the intensity of the fluorescence signals from the exposed wells by the intensity of signals from the control wells and multiplying the result by 100. These ratios were then used to construct dose-response curves, from which IC_{50} values and the concentrations of the respective compounds that were lethal to 50% of the tumor cells, were calculated. Estrone and 17β-estradiol were used as controls.

Acknowledgments

We are indebted to Dr. Vašíčková for taking the IR spectra and Dr. P. Fiedler for interpreting them. The optical rotations were measured and elemental analyses were carried out in the Analytical Laboratory (Dr. S. Matějková, Head). The technical assistance of Mrs. D. Hybšová and O. Hustáková is gratefully acknowledged. This work was supported by projects MSMT 1M06030, GAAV IAA400550801, RVO 61388963, and ED0007/01/01 from the Centre of the Region Haná for Biotechnological and Agricultural Research.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.10.013.

References and notes

1. Rosenberg, B.; Van Camp, L.; Krigas, T. *Nature* **1965**, *205*, 698.
2. Rosenberg, B.; Van Camp, L.; Grimley, E. B.; Thomson, A. J. *J. Biol. Chem.* **1967**, *242*, 1347.
3. Sbovata, S. M.; Bettio, F.; Mozzon, M.; Bertani, R.; Venzo, A.; Benetollo, F.; Michelin, R. A.; Gandin, V.; Marzano, C. *J. Med. Chem.* **2007**, *50*, 4775.
4. Kovala-Demertzi, D.; Galani, A.; Kourkoumelis, N.; Miller, J. R.; Demertzis, M. A. *Polyhedron* **2007**, *26*, 2871.
5. Liu, X.; Shen, H.; Zhu, H. B.; Cui, K.; Gou, S. H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3831.
6. Zhang, J. C.; Zhao, X. J. *Eur. J. Med. Chem.* **2007**, *42*, 286.
7. Abu-Surrah, A. S.; Kettunen, M. *Curr. Med. Chem.* **2006**, *13*, 1337.
8. Momekov, G.; Bakalova, A.; Karaivanova, M. *Curr. Med. Chem.* **2005**, *12*, 2177.
9. Boulikas, T.; Vougiouka, M. *Oncol. Rep.* **2003**, *10*, 1663.
10. Wang, X. *Anti-Cancer Agents Med. Chem.* **2010**, *10*, 396.
11. Gust, R.; Beck, W.; Jaouen, G.; Schoenenberger, H. *Coord. Chem. Rev.* **2009**, *253*, 2760.
12. Bruijnincx, P. C. A.; Sadler, P. J. *Curr. Opin. Chem. Biol.* **2008**, *12*, 197.
13. Coluccia, M.; Natile, G. *Anti-Cancer Agents Med. Chem.* **2007**, *7*, 111.
14. Abu-Surrah, A. S. *Mini-Reviews in Medicinal Chemistry* **2007**, *7*, 203.
15. Fuertes, M. A.; Castilla, J.; Nguewa, P. A.; Alonso, C.; Perez, J. M. *Med. Chem. Rev.-Online* **2004**, *1*, 187.
16. Dabrowiak, J. C. *Metals in Medicine*, 1st ed.; John Wiley and Sons: Chichester, 2010.
17. Farrell, N. *Uses of inorganic chemistry in medicine*, 1st ed.; Royal Society of Chemistry: Cambridge, 1999.
18. Gandolfi, O.; Blum, J.; Mandelbaum-Shavit, F. *Inorg. Chim. Acta* **1984**, *91*, 257.
19. Fernández, J. M.; Rubio-Arroyo, M. F.; Rubio-Poo, C.; Peña, A. *Monatsh. Chem.* **1983**, *114*, 535.
20. Chesne, C.; Leclercq, G.; Pointeau, P.; Patin, H. *Eur. J. Med. Chem.* **1986**, *21*, 321.
21. Kirschner, M. A. In *Breast Cancer 3: Advances in Research and Treatment-Current Topics*; McGuire, W. L., Ed.; Plenum Press: New York, 1979; Vol. 3, pp 199–226.
22. Henderson, B. E.; Ross, R.; Bernstein, L. *Cancer Res.* **1988**, *48*, 246.
23. Panchapakesan, G.; Dhayalan, V.; Moorthy, N. D.; Saranya, N.; Mohanakrishnan, A. K. *Steroids* **2011**, *76*, 1491.
24. Li, C.; Qiu, W.; Yang, Z.; Luo, J.; Yang, F.; Liu, M.; Xie, J.; Tang, J. *Steroids* **2010**, *75*, 859.
25. Bubert, C.; Leese, M. P.; Mahon, M. F.; Ferrandis, E.; Regis-Lydi, S.; Kasprzyk, P. G.; Newman, S. P.; Ho, Y. T.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **2007**, *50*, 4431.
26. Saxena, H. O.; Faridi, U.; Kumar, J. K.; Luqman, S.; Darokar, M. P.; Shanker, K.; Chanotiya, C. S.; Gupta, M. M.; Negi, A. S. *Steroids* **2007**, *72*, 892.
27. Motylewska, E.; Mełeń-Mucha, G. *J. Steroid Biochem. Mol. Biol.* **2009**, *113*, 75.
28. Schobert, R.; Seibt, S.; Effenberger-Neidnicht, K.; Underhill, C.; Biersack, B.; Hammond, G. F. *Steroids* **2011**, *76*, 393.
29. Phan, C. M.; Liu, Y.; Kim, B.; Mostafa, Y.; Taylor, S. D. *Bioorg. Med. Chem.* **2011**, *19*, 5999.
30. Gupta, A.; Saha, P.; Descôteaux, C.; Leblanc, V.; Asselin, É.; Bérubé, G. *Bioorg. Med. Chem.* **2010**, *20*, 1614.
31. Ruiz, J.; Rodríguez, V.; Cutillas, N.; Espinosa, A.; Hannon, M. J. *J. Inorg. Biochem.* **2011**, *105*, 525.
32. Provencher-Mandeville, J.; Debnath, C.; Mandal, S. K.; Leblanc, V.; Parent, S.; Asselin, É.; Bérubé, G. *Steroids* **2011**, *76*, 94.
33. Huxley, M.; Sanchez-Cano, C.; Browning, M. J.; Navarro-Ranninger, C.; Quiroga, A. G.; Rodger, A.; Hannon, M. J. *Dalton Trans.* **2010**, *39*, 11353.
34. Yaya, A. R.; Touaibia, M.; Massarweh, G.; Rochon, F. D.; Breau, L. *Steroids* **2010**, *75*, 489.
35. Kvasnica, M.; Budesinsky, M.; Swaczynova, J.; Pouzar, V.; Kohout, L. *Bioorg. Med. Chem.* **2008**, *16*, 3704.
36. Descôteaux, C.; Leblanc, V.; Bélanger, G.; Parent, S.; Asselin, É.; Bérube, G. *Steroids* **2008**, *73*, 1077.
37. Schobert, R.; Bernhardt, G.; Biersack, B.; Bollwein, S.; Fallahi, M.; Grotemeier, A.; Hammond, G. L. *ChemMedChem* **2007**, *2*, 233.
38. Perron, V.; Rabouin, D.; Asselin, É.; Parent, S.; Gaudreault, R. C.; Bérubé, G. *Bioorg. Chem.* **2005**, *33*, 1.
39. Criado, J. J.; Manzano, J. L.; Rodríguez-Fernández, E. *J. Inorg. Biochem.* **2003**, *96*, 311.
40. Gabano, E.; Cassino, C.; Bonetti, S.; Prandi, C.; Colangelo, D.; Ghiglia, A.; Osella, D. *Org. Biomol. Chem.* **2005**, *3*, 3531.
41. Jackson, A.; Davis, J.; Pither, R. J.; Rodger, A.; Hannon, M. *J. Inorg. Chem.* **2001**, *40*, 3964.
42. Nilsson, S.; Mäkelä, S.; Treuter, E.; Tujague, M.; Thomsen, J.; Andersson, G.; Enmark, E.; Pettersson, K.; Warner, M.; Gustafsson, J. *Physiol. Rev.* **2001**, *81*, 1535.
43. Reddel, R. R.; Sutherland, R. L. *Cancer Res.* **1987**, *47*, 5323.
44. Provencher-Mandevile, J.; Descoteaux, C.; Mandal, S. K.; Leblanc, V.; Asselin, É.; Bérubé, G. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2282–2287.