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Studies on organometallic selective estrogen receptor modulators. (SERMs) Dual activity in the hydroxy-ferrocifen series

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Abstract

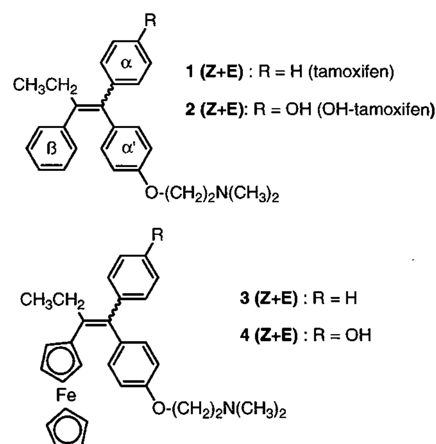
Synthesis of **7**, a ferrocene derivative of the antiestrogenic drug hydroxytamoxifen bearing a basic chain-$O(CH_2)_nN(CH_3)_2$ with $n = 4$ is presented, together with both studies of its antiproliferative effect on the hormone-dependent MCF7 cell line (estrogen receptor positive cells) and of its genotoxicity. This molecule is easily prepared via a McMurry coupling reaction. The antiproliferative effect found for **7** at an incubation molarity of 1 μM was very close to that found for the usual reference molecule, namely OH-tamoxifen. In addition to its structural antiestrogenic effect, **7** showed cytotoxic activity probably due to the vectored ferrocene. This genotoxic component was confirmed by a 3D (damaged DNA detection) test, that permits identification and quantification of lesions induced on DNA. Some key interactions of **7** docked into the alpha-estrogen receptor binding site were also shown. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Organometallic hormone; Ferrocene; Antiestrogen; Tamoxifen; MCF7; Cytotoxicity

1. Introduction

The personal and social costs of breast cancer are well documented [1]. To date, other than surgery and radiotherapy, the therapeutic arsenal consists chiefly of antiestrogenic selective estrogen receptor modulators (SERMs), of which tamoxifen **1** remains the primary example (Scheme 1) [2], as well as inhibitors of the aromatase, the key enzyme of the biosynthesis of estradiol in women, taxol derivatives and specific monoclonal antibodies again breast tumors, plus the chemical cocktails of classic chemotherapy. Despite their proven utility, the very multiplicity of these approaches attests to the failure to find a truly successful therapeutic solution. Research in this area in fact remains very active and has recently benefited from important advances in receptorology.

Some significant recent developments concern the estrogen receptor and its mode of activity. First, it has been found that there are actually two forms of the estrogen receptor, the long-known α form (ERα) and also a β form (ERβ), both implicated in breast cancer,



Scheme 1.

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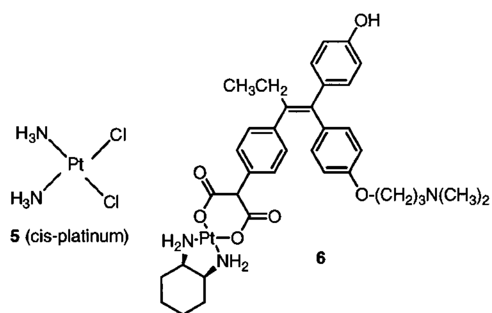
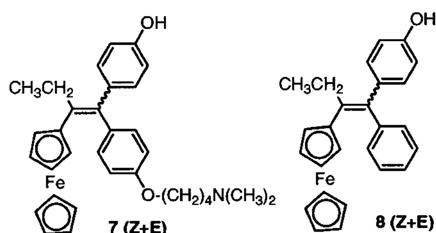


Scheme 2.



Scheme 3.

although to differing degrees [3,4]. Secondly, the structure of the ligand binding domain (LBD) of these two receptors attached to various bioligands has been published [5–8]. Finally, two activation pathways modulating the hormonal effect at the DNA level have been identified, namely the estrogen response element (ERE) and activator protein 1 (AP1) [4,9]. These discoveries add a new level of complexity to our understanding of regulatory phenomena in the body, but for the first time they also make it possible to think in molecular terms about this hormone-dependent cancer, and they present interesting new challenges to the chemist.

It is a fact that the standard SERM, tamoxifen (**1**), is only effective on approximately 60% of tumours, those classified estrogen receptor positive (ER +). Tamoxifen also causes resistance over the long term and has undesirable side effects, chiefly endometrial or thromboembolic in nature [9]. Although no ideal SERM exists, a considerable improvement would be a molecule active against both ER + and ER − (estrogen receptor negative) tumors. This can only be achieved by taking a new therapeutic approach. We present here some of our results in this area, based on the particular properties of ferrocene chemistry.

2. Background to the problem

Inorganic platinum complexes, of which the archetype is cis-platinum, **5** (Scheme 2), have a well-established status as effective antitumoral agents, despite a relatively narrow therapeutic range (testicular, urogenital, head, neck and colon cancers) [10–12]. One of the most spectacular success stories to come from these compounds is the case of testicular cancer which used to have a very high mortality rate and is now very much a curable condition. This breakthrough led to research on organometallic compounds of various metals for antitumoral applications [13,14]. Of these, the cyclopentadienyl complexes of Ti, Fe, Mo, V, Re have given encouraging results, although no compounds of this type have as yet completed clinical trials.

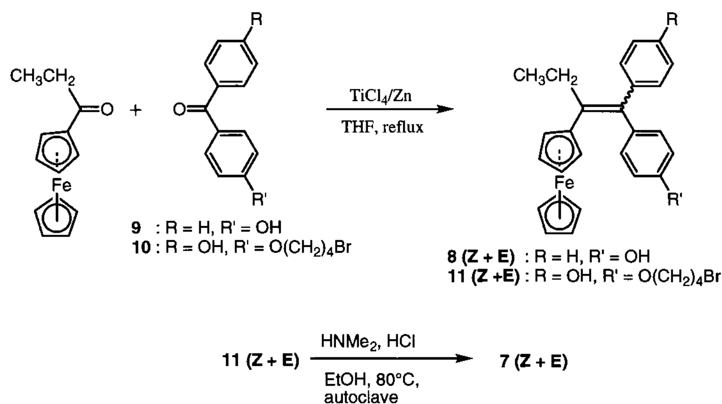
It appears however that these metallocenes operate via different mechanisms than cis-platinum [13,14] and could be used for types of tumors on which the latter is not effective. The idea of using a hormonal vector to deliver cis-platinum at the level of the estrogen receptor has been the subject of several studies [15] but, as with compound **6**, the antitumoral effects on breast cancers proved disappointing [16]. This led us to adjust the strategy and explore the potential in this area of various organometallic groups with possible cytotoxicity, attached to known SERMs so as to potentialize their effects. We have suggested previously that fixation of a ferrocenyl group onto an antiestrogen vector of the OH–tamoxifen **2** type could provide a useful modification of this SERM [17,18], but it appears that in this case the length of the carbon chain significantly perturbs its effects. We present here syntheses, biochemical studies, molecular modeling and a suggested mechanism to support the unusual behavior of hydroxyferrocifen such as **7** with a four-carbon chain $-O-(CH_2)_4NMe_2$ (Scheme 3) as compared to its equivalent **8** without basic chain.

Compound **7** and other similar ones that contain a ferrocene substituent in fact possess the unusual property of combining both antiproliferative effects on ER + breast cancer cell lines, due to a change in the conformation of the α receptor, and genotoxic effects due to the attached organometallic moiety.

2.1. Synthesis of 7 and 8

The McMurry coupling reaction ($TiCl_4$, Zn) is still the preferred method for synthesis of tamoxifen analogs [19–21]. It is easy to carry out and results in a mixture of *Z* and *E* isomers. Scheme 4 shows the synthetic routes to the ferrocene derivatives of tamoxifen.

Propionylferrocene was obtained by acylation of ferrocene via a Friedel–Crafts reaction. Stirring the propionylferrocene mixture with hydroxybenzophenone **9** in the presence of the McMurry reagent gives the dissymmetric monohydroxylated complex **8** as the major product in 36% yield. The synthesis of ferrocifen **7** begins with a coupling reaction between propionylferrocene and the ketone **10**, obtained previously by addition of the bromobutyl chain onto dihydroxybenzo-



Scheme 4.

phenone in the presence of sodium ethoxide. This gives the halogenated compound **11** which then reacts with dimethylamine chlorhydrate in an autoclave to give ferrocifen **7** as the final product in 34.4% yield.

Compounds **7** and **8** were obtained as a mixture of *Z* and *E* isomers. Thin-layer chromatography cannot be used to separate the two isomers. However separation was possible in the case of **3**, ferrocifen with a two-carbon side-chain [21] and the corresponding OH–ferrocifen **4** [18]. Isomerization leading to interconversion of the *Z* and *E* isomers is a well-known phenomenon in the stilbene series, for example in the case of diethylstilbestrol (DES) [22]. An isomerization study was performed on compound **4** at room temperature [23]. We found that **4**-*E* is indeed subject to isomerization, the speed of isomerization depending on the nature of the solvent. In chloroform the transformation is rapid, with 50% conversion after one hour in solution, while it takes a day to reach this value in acetone, and in ethanol only 30% isomerization has occurred after 24 h. In any case, this phenomenon of isomerization avoids the lengthy process of separating the two isomers.

2.2. Biochemical data

2.2.1. Determination of RBA value and lipophilicity

The study of the biochemical properties of the compounds involves first of all the determination of their relative binding affinity for estradiol receptor α. The values found for **7** and **8** are 12 and 5%, respectively, which is an acceptable level of recognition although lower than the value found for **2** (38.5%). Lipophilicity measured by the octanol/water partition coefficient gave values for **7** of 3.95 for the *Z* isomer and 4.18 for *E*. These are higher than those found for OH–tamoxifen **2** (3.17 and 3.36), which confirms that insertion of an organometallic moiety increases the lipophilicity of the hormone thus permitting better penetration of these complexes into cells.

2.3. Study of the proliferative/antiproliferative effect on MCF7 cells

The proliferative/antiproliferative effect of complexes **7** and **8** and of the ferrocene (Fc) entity alone were studied on the hormone-dependent MCF7 cell line (estrogen receptor positive cells). The results obtained are shown in Fig. 1. They are expressed as a percentage of DNA compared to control (CTR; cells incubated in the presence of the vehicle). For estradiol (E_2), used as a standard for the proliferative effect, a value of 186% was found, while OH–tamoxifen **2**, standard for the antiproliferative effect, gave a value of 35%. For ferrocifen **7**, at an incubation molarity of 1 μM, an antiproliferative effect very close to that of **2** is found. As well, this effect is only partially inhibited by addition of estradiol into the medium showing that the observed effect can be divided into two components, the first

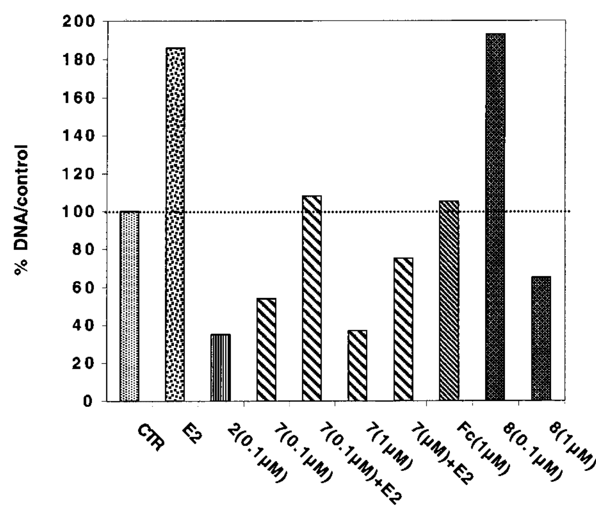


Fig. 1. Effect of E_2 (estradiol; 1×10^{-9} M), **2** (OH–tamoxifen), Fc (ferrocene) and ferrocene derivatives **7** and **8** on the proliferation of MCF7 cells (estrogen receptor-positive cells). The results are expressed as the percentage of DNA in the sample versus the DNA value of the control (CTR).

anti-hormonal, due to the presence of the dimethylamino chain, and the other cytotoxic, probably caused by the ferrocene entity whose cytotoxic effect on certain cell lines has already been reported [14]. In the present case, however, the effect is not entirely due to the ferrocene entity, since incubation of this entity has no effect on MCF7 cells. The addition of a vector is thus required. It should be noted as well that at a molarity of 0.1 μM only an antagonist effect is seen in **7** (with complete inhibition of the effect by addition of E_2). Moreover, incubation in the presence of **8**, **7** without the antiestrogenic chain, shows that at an incubation molarity of 0.1 μM, **8** behaves as expected as an estrogen (186%) while at a molarity of 1 μM it becomes cytotoxic for the cells (65%), although still less so than the ferrocifen **7**.

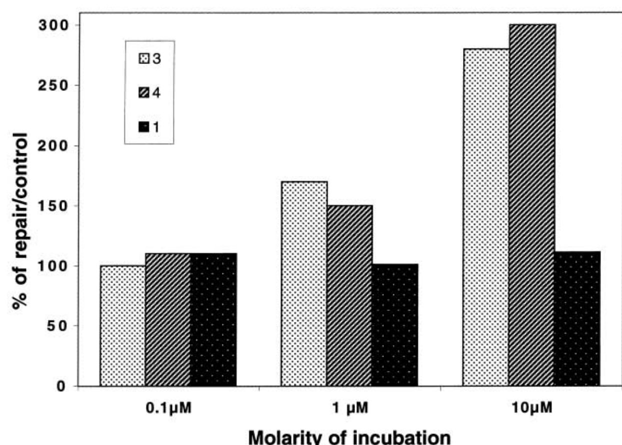


Fig. 2. Dose effect of **1**, **3** and **4** on 3D assay (DNA, damaged, detection). Target DNA is incubated for 30 min at 30 °C in the presence of various concentration of the molecules to be tested. The results are expressed as the percentage of repair of the sample under study vs. the value of control. For details see text and Ref. [25].

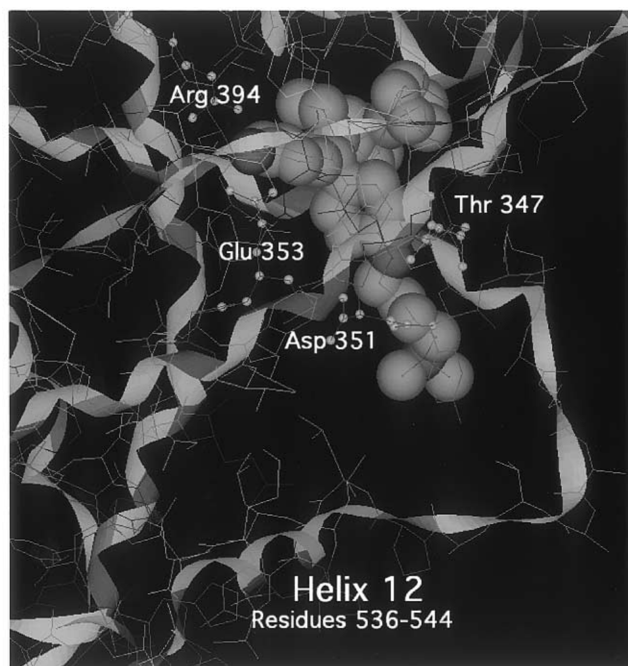


Fig. 3. Some key interactions of OH–ferrocifen **7** docked into the alpha-estrogen receptor binding site. This study was performed by using the structural basis of the ER ligand binding domain published by Shiau et al. [7] and the MOLVIEW program designed by Cense [26].

2.4. 3D assay (damaged DNA detection)

The increased cytotoxic effect of ferrocene derivatives seen in MCF7 cells beginning at micromolar concentrations is entirely corroborated by the 3D assay, patented recently and published by the company SFRI [24,25]. This test permits identification and quantification of lesions induced on DNA (oxidative lesions, formation of adducts) as well as the effect of molecules on the modulation of the reparative activity of the DNA from the cell extract used in the test. Quantitative evidence of the number of lesions repaired is obtained by chemiluminescence. Using this test, we compared tamoxifen **1** and their ferrocene equivalents **3** and **4** (Fig. 2). Values are shown as percentages of lesions compared to control (100%). For a given molecule, a value above 100%, therefore, shows that lesions were induced on DNA. The results obtained show that ferrocifen **3** and its corresponding hydroxylated form **4** cause a similar rate of lesions on DNA. The first significant effects are observed at a molarity of 1 μM, and they become very pronounced at a molarity of 10 μM. In the same conditions, tamoxifen **1** has no effect. Moreover, none of the compounds tested, **1**, **3**, **4**, inhibited the reparative capacity of the lesions (data not shown). Finally, UV-light irradiation of the compounds under 3D test conditions had no effect. These results corroborate those obtained by incubation of MCF7 cells in the presence of ferrocifens: a cytotoxic effect that becomes apparent at an incubation molarity of 1 μM, and death of the cells at an incubation molarity of 10 μM.

3. Discussion

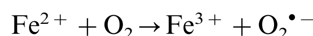
The results obtained here with ferrocene SERMs that are tamoxifen analogs show a similarity in behavior between these two species in their structural antagonist effect against ERα, and at the same time they also reveal an intrinsic cytotoxic component due to the organometallic entity.

The X-ray crystal structure of ERα contained in the MCF7 cell lines, with hydroxytamoxifen **2** attached at the ligand binding domain, has been published [7]. Taking this work as our basis, we used MOLVIEW [26] to model the antagonist site and introduced hydroxyferrocifen **7** into the active site. The result is shown in Fig. 3. As with **2**, the phenol is still associated with Arg 394

(distance 2.54 Å) and Glu 353 (2.73 Å) residues, and the ferrocene entity that replaces the benzene ring β goes into place without disturbing the protein at the level of histidine 524. The basic chain is responsible in these compounds for the primary antagonistic effect, as it changes the position of helix 12 relative to its agonist conformation, and thus inhibits the fixation of the coactivators at this level (residues 536–544). The distance between the Asp 351 and $-N(CH_2)_3$ residues and the chain (5.35 Å) appears too great to be compatible with the strong stabilizing association which has been observed in the model in the presence of raloxifen [5], but not with **2** [7].

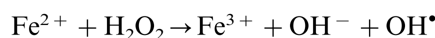
On the other hand, the addition of a ferrocene substituent introduces an oxidizing/reducing genotoxic aspect that is absent in the organic equivalents. This effect can be seen at micromolar concentrations. The study of the antiproliferative effects on the MCF7 cell line and the observation of the genotoxic effect of ferrocene complexes as seen in the 3D test are in agreement on this subject. Both tests reveal this unusual behavior which is due to the vectored ferrocene.

It has been reported widely in the literature that the cytotoxic effect of ferrocene complexes is associated with their oxidation to ferricinium-type radical ions [14] and we have checked by cyclic voltammetry that the potential standard of **7** was very closed to that of ferrocene alone (0.474 and 0.460 V, respectively). Until recently, the reason for their ability to cleave DNA was not clear and could be linked either to a direct association or to oxidative damage. A study by Tamura has shown that this cytotoxic activity is due to the generation of $OH^{\cdot}$ [27]. Recent work by Osella et al. also militates in favor of there being no direct ferricinium–DNA interaction, but instead the generation of active oxygenated species [28]. The results of the 3D test bear out this analysis. It can in fact be seen that, unlike tamoxifen **1**, the corresponding ferrocene complexes **3** and **4** show genotoxic activity in this test. Moreover, this activity occurs by production of lesions on DNA but does not seem to interfere with the reparative phenomena, since repair is not inhibited (data not shown) as could have been the case with an adduct. This effect can be compared to the Fenton reaction:



$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\bullet -}$$

$$Fe^{2+} + O_2^{\bullet -} + 2H^+ \rightarrow Fe^{3+} + H_2O_2$$



$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet}$$

It is known that the $O_2^{-\bullet}$ radical shows very low reactivity for DNA, but that the $OH^{\bullet}$ radical is highly reactive, causes various types of lesions and is thus very genotoxic.

4. Conclusions

The dual activity of the ferrocene complexes of tamoxifen on ERα-containing MCF7 cells points to interesting new directions in the search for SERMs that will be more effective against breast cancer. It has been hypothesized that the spread of breast cancers that evade the effects of tamoxifen often involves ERβ and the AP1 regulation pathway [3,9]. Tamoxifen then behaves as an agonist and becomes ineffective. But ERβ is also implicated in cell oxido-reduction [29]. This leads one to consider that the phenomenon described here might be particularly useful in the control of antiproliferative effects with ERβ-containing breast cancer cells. This work is underway.

5. Experimental

5.1. General remarks

The synthesis of all the compounds was performed under an argon atmosphere, using the Schlenk line technique and Schlenk flasks. Anhydrous THF and anhydrous diethyl ether were obtained by distillation from sodium/benzophenone. 4-Hydroxybenzophenone (**9**) and 4,4-dihydroxybenzophenone were purchased from Aldrich. Ferrocenyl ethyl ketone was prepared according the procedure described in Ref. [32]. TLC chromatography was performed on silica gel 60 GF254. ^1H- and ^{13}C-NMR spectra were recorded on 200 MHz and 250 MHz Bruker spectrometers. Mass spectrometry was performed with a Nermag R 10-10C spectrometer. Melting points were measured with a Kofler device. Elemental analysis was performed by the regional microanalysis service of Université Pierre et Marie Curie.

*5.1.1. Synthesis of 1-(4-hydroxyphenyl)-1-(phenyl)-2-ferrocenyl-but-1-ene (**8**(Z + E))*

$TiCl_4$ (6.58 ml, 60 mmol) was added dropwise to a suspension of zinc powder (7.80 g, 120 mmol) in THF (90 ml) at −10 °C. The dark grey mixture obtained was heated at reflux for 1.5 h and then allowed to cool to room temperature (r.t.). A solution of THF (10 ml) containing 4-hydroxybenzophenone (**9**) (3.96 g, 20 mmol) and ferrocenyl ethyl ketone (4.84 g, 20 mmol) were added dropwise to the first solution and then the resulting mixture was heated for 2 h. After cooling to r.t., the mixture was hydrolyzed with 10% K_2CO_3 solution. After ether extraction and solvent removal, the crude product was chromatographed on a silica gel column with ethyl ether/pentane 1:2 as eluent. 2.93 g of 1-(4-hydroxyphenyl)-1-(phenyl)-2-ferrocenyl-but-1-ene (**8**(Z + E)) were isolated, orange solid, 36% (m.p. 161 °C). ^1H-NMR (200 MHz, DMSO-d_6) δ 9.33 (s, 1H OH), 7.33–6.63 (m, 9H, C_6H_5 + C_6H_4), 4.12, and 4.11

(s, s, 5H, Cp),), 4.09, 4.05, 3.82, 3.76 (t, t, t, t, 4H, C_5H_4), 2.50 (m, 2H, CH_2), 1.00 and 0.99 (t, t, 3H, CH_3). Anal. Calc. for $C_{26}H_{24}OFe$: C, 76.48; H, 5.92. Found: C, 76.39; H, 5.90%.

*5.1.2. 1-[4-(4-Dimethylaminobutoxy)phenyl]-1-(4-hydroxyphenyl)-2-ferrocenyl-but-1-ene (**7**(Z + E))*

Dihydroxybenzophenone (2.14 g, 10 mmol) was added to a solution of sodium ethanolate prepared by treating sodium (0.230 g, 10 mmol) with ethanol (15 ml). After stirring at reflux for 1 h, 1,4-dibromobutane (10.80 g, 50 mmol) was added. After 1 h of reflux, the solution was left to cool to r.t. and hydrolyzed with 100 ml water. The product was extracted with dichloromethane. The organic phase was washed with water, dried over magnesium sulfate, filtered and the solvent evaporated. The crude product was chromatographed on silica gel plates with ethyl ether/pentane 2:1 as eluent. 4,4′-di(4-Bromobutoxy)benzophenone compound was first isolated as a white solid, 0.133 g (27% yield). NMR ^1H (200 MHz, acetone-d_6) δ 7.74 (d, 4H, C_6H_4–O); 7.06 (d, 4H, C_6H_4–O); 4.17 (t, 4H, OCH_2); 3.61 (t, 4H, CH_2Br); 2.03 (m, 8H, CH_2–CH_2). m.p. 111 °C. The second compound was isolated as a yellow oil which solidified in pentane and identified as **10**, 1.005 g, 28% yield. NMR ^1H (200 MHz, acetone-d_6) δ 7.74 (d, 2H, C_6H_4–O); 7.68 (d, 2H, C_6H_4–O); 7.05 (d, 2H, C_6H_4–O); 6.96 (d, 2H, C_6H_4–O); 4.16 (t, 2H,OCH_2); 3.61 (t, 2H, CH_2Br); 2.03 (m, 4H, CH_2CH_2). M.p. 98 °C, white crystals.

The coupling reaction between ferrocenyl ethyl ketone and **10** is similar to that of ferrocenyl ethyl ketone and **9**. $TiCl_4$ (0.346 g, 1.82 mmol), zinc powder (0.195 g, 2.98 mmol), 10 (0.150 g, 0.5 mmol), ferrocenyl ethyl ketone (0.121 g, 0.5 mmol). After a work up, the crude product, 0.374 g, was chromatographed on silica gel plates with ethyl ether/pentane 1:1 as eluent to yield **11** (0.110 g, 46%) as an orange solid, m.p. 57 °C. NMR ^1H (200 MHz, $CDCl_3$) 6.85 (m, 8H, C_6H_4–O); 4.06 (s, 5H, C_5H_5); 3.98 (t, 2H,OCH_2); 3.94 (d, 1H, C_5H_4); 3.90 (d, 1H, C_5H_4); 3.46 (t, 2H, C_5H_4); 3.46 (t, 2H, CH_2Br); 2.58 (q, 2H, $\mathit{CH_2}CH_3$); 1.01 (t, 3H, $CH_2\mathit{CH_3}$).

Subsequently, **11** (0.150 g, 0.27 mmol), $HNMe_2$·HCl, (0.170 g), NEt_3 (0.2 ml), and ethanol (25 ml) were placed in an autoclave. The mixture was heated at 110 °C for 6 h, the solvent evaporated and the crude product obtained chromatographed on Silicagel plates with acetone/NEt_3: 20/1 as eluent to yield **7** (0.047 mg; 34.4%) as an orange solid, m.p. 59 °C. NMR ^1H (200 MHz, DMSO) δ 9.32 (s, 1H, OH); 7.10–6.61 (m, 8H, C_6H_4–O); 4.10 (s, 5H, C_5H_5); 4.08 (m, 2H, C_5H_4); 3.81 (m, 2H, C_5H_4); 3.93 (dd, 2H, OCH_2); 2.37 (m, 2H, $-\mathit{CH_2}NMe_2$); 2.22 (s, 6H, NMe_2); 1.60 (m, 4H, $-CH_2CH_2-$); 0.98(t, 3H, $CH_2\mathit{CH_3}$). Mass spectrum (EI, 70 eV) m/z: 523 [M^+], 100 [$(CH_2)_4NMe_2^+$]. Anal. Calc. for $C_{32}H_{37}NO_2Fe.H_2O$: C, 70.98; H, 7.26; N, 2.58. Found: C, 70.23; H, 7.12; N, 2.86%.

*5.2. Determination of the RBA of **7** and **8** for the estrogen receptor alpha (ERα)*

Aliquots (200 μl) of sheep uterine cytosol prepared as described in Ref. [30] were incubated for 3 h at 0 °C with [6,7-^3H]-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 [^3H]-estradiol (Y) was calculated using the logit transformation of Y (logitY: $\ln[y/1-Y]$ versus the log of the mass of the competing steroid. The concentration of unlabeled steroid required to displace 50% of the bound [^3H]-estradiol was calculated for each steroid tested, and the results expressed as RBA. The RBA value of estradiol is by definition equal to 100%.

5.3. Test on MCF7 cells

5.3.1. Culture materials

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

5.3.2. Culture conditions

MCF7 cells were from the Michigan Cancer Foundations (Detroit). Cells are maintained in monolayer culture in Dulbecco-MEM added with 10% thermically inactivated FBS, L-glutamine (0.6 mg ml^{-1}) and a cocktail of antibiotics (gentamicin 40 μg ml^{-1}, penicilin 100 U ml^{-1}, streptomycin 100 μg ml^{-1}). The growth of the cells was assessed by measuring the DNA content of treated and untreated (control) cells after 120 h of culture [31].

Acknowledgements

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References

[1] E.J. Feuer, L.M. Wun, C.C. Boring, W.D. Flanders, M.J. Timmel, T. Tong, J. Natl. Cancer Inst. 85 (1993) 892.
[2] J.I. Mac Cregor, V.C. Jordan, Pharmacol. Rev. 50 (1998) 151.
[3] J. Gustafsson, J. Endocr. 163 (1999) 379.
[4] K. Paech, P. Webb, G.G.J.M. Kuiper, S. Nilsson, J.-A. Gustafsson, P.J. Kushner, T.S. Scanlan, Science 277 (1997) 1568.

[5] A.M. Brzozowski, A. Pike, Z. Dauter, R.E. Hubbard, T. Bonn, O. Engstrom, L. Ohman, G. Greene, J.-A. Gustafsson, M. Carlquist, Nature 389 (1997) 753.
[6] A.C. Pike, A.M. Brzozowski, R.E. Hubbard, T. Bonn, A.G. Thorsell, O. Engstrom, J. Ljunggren, J.-A. Gustafsson, M. Carlquist, EMBO 18 (1999) 4608.
[7] A.K. Shiau, D. Barstad, P.M. Loria, L. Cheng, P.J. Kushner, D.A. Agard, G. Greene, Cell 95 (1998) 927.
[8] M. Tanenbaum, Y. Wang, S.P. Williams, P.B. Sigler, Proc. Natl. Acad. Sci. USA 95 (1998) 5998.
[9] K. Dhingra, Invest. New Drugs 17 (1999) 285.
[10] Z. Guo, P.J. Sadler, Angew. Chem. Int. Ed. Engl. 38 (1999) 1512.
[11] M.E. Heim, in: B.K. Keppler (Ed.), Metal Complexes in Cancer Chemotherapy, VCH, Weinheim, 1993.
[12] B. Rosenberg, Interdisciplinary Sci. Rev. 3 (1978) 134.
[13] P. Köpf-Maier, in: B.K. Keppler (Ed.), Metal Complexes in Cancer Chemotherapy, VCH, Weinheim, 1993, p. 259.
[14] P. Köpf-Maier, Eur. J. Clin. Pharmacol. 47 (1994) 1.
[15] E. Von Angerer, in: B.K. Keppler (Ed.), Metal Complexes in Cancer Chemotherapy, VCH, Weinheim, 1993, p. 73.
[16] S. Top, unpublished results.
[17] G. Jaouen, S. Top, A. Vessières, G. Leclercq, J. Quivy, L. Jin, A. Croisy, C. R. Acad. Sci. Paris Ser. IIc 3 (2000) 89.
[18] S. Top, J. Tang, A. Vessières, D. Carrez, C. Provot, G. Jaouen, Chem. Commun. (1996) 955.
[19] P.L. Coe, C.E. Scriven, J. Chem. Soc. Perkin Trans I 3 (1986) 475.
[20] S. Gauthier, J. Mailhot, F. Labrie, J. Org. Chem. 61 (1996) 3890.
[21] S. Top, B. Dauer, J. Vaisserman, G. Jaouen, J. Organomet. Chem. 541 (1997) 355.
[22] V.W. Winkler, M.A. Nyman, R.S. Egan, Steroids 17 (1971) 197.
[23] J. Tang, PhD thesis, University Paris VI, 1995.
[24] K.S. Kasprak, Cancer Invest. 13 (1995) 411.
[25] B. Salles, C. Provot, P. Calson, I. Hennebelle, I. Gosset, G.J. Fournié, Anal. Biochem. 232 (1995) 37.
[26] J.M. Cense, Phys. Theor. Chem. 71 (1990) 763.
[27] H. Tamura, M. Miwa, Chem. Lett. (1997) 1177.
[28] D. Osella, M. Ferrali, P. Zanello, F. Lashie, M. Fontani, C. Nervi, G. Cavigiolio, Inorg. Chim. Acta 306 (2000) 42.
[29] M.M. Montano, A.K. Jaiswal, B.S. Katzenellenbogen, J. Biol. Chem. 273 (1998) 25443.
[30] A. Vessières, S. Top, A.A. Ismail, I.S. Butler, M. Loüer, G. Jaouen, Biochemistry 27 (1988) 6659.
[31] G. Leclercq, N. Devleeschouwer, J.C. Heuson, J. Steroid Biochem. 19 (1983) 75.
[32] P.J. Graham, R.V. Lindsey, G.W. Parshall, M.L. Peterson, G.M. Whitman, J. Am. Chem. Soc. 79 (1957) 3416.