Ferrocenyl hydroxytamoxifen: a prototype for a new range of oestradiol receptor site-directed cytotoxics

Siden Top,[a] Jie Tang,[a] Anne Vessières,[a] Danièle Carrez,[b] Christian Provot[c] and Gérard Jaouen*[a]†

[a] *Laboratoire de Chimie Organométallique, Ecole Nationale Supérieure de Chimie de Paris, URA CNRS 403, 11, rue Pierre et Marie Curie, 75231 Paris Cédex 05, France*

[b] *INSERM 350, Institut Curie, Section de Biologie, Bât. 112, Centre Universitaire, 91405 Orsay Cédex, France*

[c] *S.F.R.I., Berganton, 33127 Saint Jean d'Illac, France*

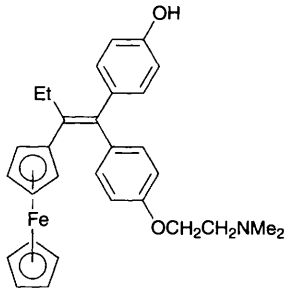
The synthesis of ferrocenyl hydroxytamoxifen 1, a prototype for a new range of oestradiol receptor site-directed cytotoxic compounds, and some preliminary biochemical tests are reported.

Breast cancer, which affects one in nine women in the Western world, is the most common type of malignancy and one of the leading causes of death in this demographic group. Recent papers indicate that this disease is now recognised as also a serious threat in man with genetical predisposition.[1] Aromatase inhibitors are well advanced as potential therapeutic agents[2] while oestrone sulfatase inhibitors are promising novel weapons against this type of disease.[3] Tamoxifen (TAM), a non-steroidal anti-oestrogen, has been shown to provide effective treatment in both pre- and post-menopausal women, at all stages of the disease.[4] As its primary action is to halt the progress of the tumour rather than to eradicate it, TAM must be used as part of a long-term therapeutic strategy. Certain problems have been linked to its use; in particular, patients tend to build up resistance to the drug over time, and eventually may develop TAM-stimulated tumours and endometrial cancers.[5] Research aimed at finding new and effective anti-oestrogens, without the disadvantages of TAM, are clearly of great importance.[6]

Given the fact that (*Z*)-4-hydroxytamoxifen (OH-TAM), with its OH group enhancing recognition of the oestradiol receptor, is the active metabolite of TAM, and the fact that ferrocene's first metabolite is the ferricinium ion, itself a proven anti-tumour agent,[7] a synthetic method for (*Z*)-Fc-OH-TAM **1** or related compounds would seem to be particularly advantageous. It would provide a means for two effects to coexist within a single molecule, and could lead to a range of compounds with both anti-oestrogen and anti-tumour properties.

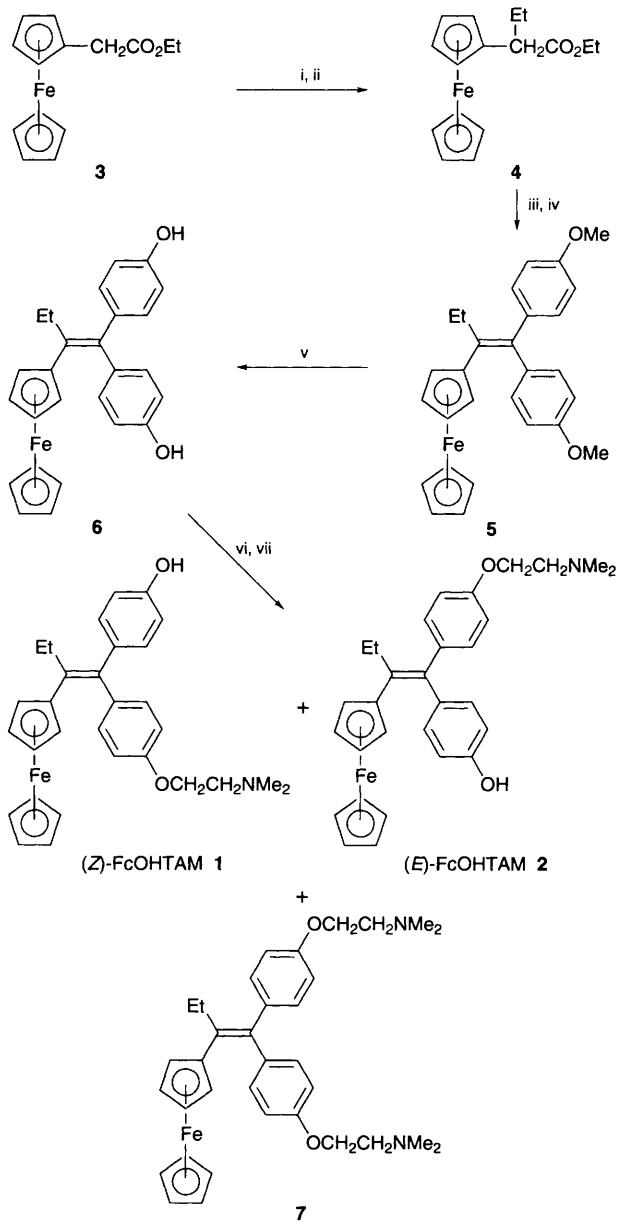
The synthesis of the (*Z*)- and (*E*)-isomers of ferrocenyl hydroxytamoxifen, **1** and **2** respectively, requires a synthetic strategy that takes into account the particular nature of ferrocene chemistry. Scheme 1 shows the approach that was adopted. The ethyl ester **3** was prepared following the literature method.[8]

The next transformation involved ethylating the α-carbon of the ferrocene ring. This was best achieved by nucleophilic attack of the anion, generated from the ethyl ester **3** by use of Bu^tOK in Me_2SO, or iodoethane. In order to avoid formation of the disubstituted compound, iodoethane must be added rapidly and hydrolysis must follow immediately. This gave ethyl 2-ethyl-2-ferrocenylacetate **4** in 47% yield. 1,1-Bis(4-methoxy-



(*Z*)-FcOHTAM **1**

Fig. 1



Scheme 1 *Reagents and conditions*: i, Bu^tOK, Me_2SO; ii, EtI; iii, 2 $MeOC_6H_4Li$; iv, H^+/H_2O; v, BBr_3, CH_2Cl_2; vi, EtONa; vii, $Me_2NCH_2CH_2Cl$, HCl

phenyl)-2-ferrocenyl-butene **5** was obtained in 63% yield by the action of $MeOC_6H_4Li$ on the ester **6**.[9] Demethylation of **5**, using 3 equiv. of BBr_3,[10] gave 1,1-bis-(4-hydroxy-phenyl)-2-ferrocenyl-butene **6** in 79% yield.

The final step was to attach the basic chain onto one of the two phenolic functions of compound **6**. The action of sodium ethoxide followed by that of 2-dimethylaminoethyl chloride produced a mixture of the (*Z*)- and (*E*)-isomers, **1** and **2**, (50:50) plus a small quantity of the disubstituted compound **7**.

After investigating various approaches, we discovered a simple and effective method to separate the diastereoisomers **1** and **2** by fractional crystallization using a 5:1 diethyl ether–hexane mixture as a solvent.

The structures of **1** and **2** have been determined by examination of the NMR spectra. **1** and **2** were identified, respectively, as the (*Z*)- and (*E*)-isomer according to the fact that the aromatic ring located between the ferrocenyl and the second aromatic ring is subjected to a shielding effect. For example, in the case of the C_6H_4OH ring, the values change from δ 6.97 and 6.71 in the case of compound **1**, to δ 6.80 and 6.63 in the case of compound **2**.

The Relative Binding Affinity (RBA) for the oestradiol receptor was measured for the (*Z*)- and (*E*)-isomers of FcOHTAM **1** and **2**, as well as for (*Z*)-OHTAM and the (*Z* + *E*) OHTAM mixture. In order to minimize the isomerization caused by the alcohol, stock solutions of the compounds to be tested were made in Me_2SO (final concentration of Me_2SO in the biological medium: 5%). The RBA values obtained are reported in Table 1.

The relative recognition of the ferrocene compounds by the oestradiol receptor is good for both compounds but superior for the (*Z*)-compound. Better recognition of the (*Z*)-isomer is also observed in the tamoxifen series.[12] The presence of a ferrocene group, which is bulkier than a phenyl group, explains the difference in affinity between **1** and OHTAM.

These high RBA values encouraged us to make a preliminary test of cytotoxicity for these complexes. This was performed on a human cell line derived from a breast cancer and possessing oestradiol receptor sites (MCF7 ATCC). These cells are a classic model for study of oestrogen derivatives.[13] The inhibitory values (IC_{50}), corresponding to the molar amount of the compound required to destroy 50% of the cells, are 3.4 and 4.9 μmol dm^{-3} for **1** and **2** respectively. Tamoxifen under the same conditions has an IC_{50} of 6.4 μmol dm^{-3}.

Furthermore, recently a new assay called 3D (damaged DNA detecton) has been published that permits a quick and easy assessment of DNA damage induced by genotoxic compounds.[14] The preliminary results show a dramatic difference between **1**, the mixture of **1** and **2** and tamoxifen as a control. While the ferrocenic molecules exhibit a clear genotoxic activity at micromolar concentrations, tamoxifen does not present, as expected, any effect under the same conditions.[15]

Table 1 Relative binding affinity (RBA) of the compounds for the oestradiol receptor[a]

Compound	RBA (%)	Compound	RBA (%)
Oestradiol	100[b]		
(*Z*)-OHTAM	107	(*Z*)-FcOHTAM **1**	40
(*Z*) + (*E*)-OHTAM	38.5	(*E*)-FcOHTAM **2**	12

[a] The RBAs for the estrogen receptor were determined in a competition radioreceptor binding assay using lamb uterine cytosol as a source of receptor, [^{3}H]oestradiol as a tracer and an incubation period of 3 h at 0 °C.[11] [b] Value by definition.

The new FcOHTAM series has thus been shown to have a better cytotoxic effect than TAM due to the presence of the ferrocenyl group but the persistence of the antagonist effect in **1** remains unchecked. This very encouraging result suggests that complementary studies, at the biochemical level as well as in terms of new syntheses, are clearly warranted.

We thank 4M for financial support, Dr A. Croisy for cytotoxic tests, A. Cordaville for technical assistance, Besins Iscovesco for the TAM and (*Z*)-OHTAM and Barbara McGlinchey for translating the manuscript.

Footnotes

† E-mail Jaouen@ext.jussieu.fr

‡ *Spectroscopic data of* **1** *and* **2**. For **1**: ^{1}H NMR (200 MHz, Me_2SO-[$^2H_6$]) δ 9.34 (s, 1 H, OH), 6.97 and 6.71 (dd, 4 H, *J* 8.7 Hz, C_6H_4-OH), 6.89 and 6.80 (dd, 4 H, *J* 8.5 Hz, $C_6H_4OCH_2$), 4.11 (s, 5 H, C_5H_5), 4.07 (m, 2 H, C_5H_4), 3.99 (t, 2 H, *J* 6.0 Hz, OCH_2), 3.80 (m, 2 H, C_5H_4), 2.60 (t, 2 H, *J* 6.0 Hz, NCH_2), 2.49 (partially obscured by signals from Me_2SO, 2 H, CH_2CH_3), 2.20 (s, 6 H, NMe_2), 0.98 (t, 3 H, *J* 7.3 Hz, CH_2CH_3). For **2**. ^{1}H NMR (200 MHz, Me_2SO-[$^2H_6$] δ 9.29 (s, 1 H, OH), 7.08 and 6.89 (dd, 4 H, *J* 8.5 Hz, $C_6H_4OCH_2$), 6.80 and 6.63 (dd, 4 H, *J* 8.4 Hz, C_6H_4OH), 4.11 (s, 5 H, C_5H_5), 4.08 (m, 2 H, C_5H_4), 4.03 (t, 2 H, *J* 6.0 Hz, OCH_2), 3.82 (m, 2 H, C_5H_4), 2.64 (t, 2 H, *J* 6.0 Hz, NCH_2), 2.49 (partially obscured by signals from Me_2SO, 2 H, CH_2CH_3), 2.23 (s, 6 H, NMe_2), 0.98 (t, 3 H, *J* 7.3 Hz, CH_2CH_3).

References

1 S. R. Wolman, J. Sanford, S. Ratner and P. J. Dawson, *Mod. Pathol.*, 1995, **8**, 239; W. D. Thompson, *Cancer*, 1994, **74**, 279.
2 R. W. Brueggemeier, *Cancer*, 1994, **30**, 31; P. E. Goss and K. M. Gwyn, *J. Clin. Oncol.*, 1994, **12**, 2460.
3 J. R. Pasqualini, G. Chetrite, B. L. Nguyen, C. Maloche, L. Delalonde, M. Talbi, M. C. Feintein, C. Blacker, J. Botella and J. Paris, *J. Steroid Biochem. Mol. Biol.*, 1995, **53**, 407; J. R. Pasqualini, C. Maloche, M. Maroni and G. Chatrite, *Anticancer Res.*, 1994, **14**, 1589; H. Birnbock and E. Von Angerer, *Biochem. Pharmacol.*, 1990, **39**, 1709.
4 V. C. Jordan and C. S. Murphy, *Endocr. Rev.*, 1991, **11**, 578; J. C. Doré, J. Gilbert, E. Bignon, A. Crastes de Paulet, T. Ojasoo, M. Pons, J. P. Raynaud and J. F. Miquel, *J. Med. Chem.*, 1992, **35**, 573; P. R. Kym, G. M. Anstead, K. G. Pinney, S. R. Wilson and J. A. Katzenellenbogen, *J. Med. Chem.*, 1993, **36**, 3910; S. G. Nayfield, J. E. Karp, L. G. Ford, F. A. Dorr and B. S. Kramer, *J. Natl. Cancer Inst.*, 1991, **23**, 1450; S. Ray, A. Tandon, I. Dwivedy, S. R. Wilson, J. P. O'Neil and J. A. Katzenellenbogen, *J. Med. Chem.*, 1994, **37**, 696.
5 S. Y. Jiang, C. J. Parker and V. C. Jordan, *Breast Cancer Res. Treat.*, 1993, **26**, 139.
6 M. G. Parker, *Breast Cancer Res. Treat.*, 1993, **26**, 131.
7 P. Köpf-Maier and H. Köpf, *Chem. Rev.*, 1987, **87**, 1137; A. Houlton, R. M. G. Roberts and J. Silver, *J. Organomet. Chem.*, 1991, **418**, 107; E. W. Neuse and F. Kanzawa, *Appl. Organomet. Chem.*, 1990, **4**, 19.
8 D. Lednicer, J. K. Lindsay and C. R. Hauser, *J. Org. Chem.*, 1958, **23**, 653.
9 H. Gilman, J. L. Towle and S. M. Spatz, *J. Am. Chem. Soc.*, 1946, **68**, 2017.
10 E. H. Vickery, L. F. Pahler and E. J. Eisenbraun, *J. Org. Chem.*, 1979, **44**, 4444.
11 A. Vessières, S. Top, A. A. Ismail, I. S. Butler, M. Loüer and G. Jaouen, *Biochemistry*, 1988, **27**, 6659.
12 D. W. Robertson, J. A. Katzenellenbogen, D. J. Long, E. A. Rorke and B. S. Katzenellenbogen, *J. Steroid Biochem.*, 1982, **16**, 1; E. Bignon, M. Pons, A. Crastes de Paulet, J. C. Doré, J. Gilbert, J. Abecassis, J. F. Miquel, T. Ojasoo and J. P. Raynaud, *J. Med. Chem.*, 1989, **32**, 2092.
13 See for example: D. Coradini, A. Biffi, V. Cappelletti and G. Di Fronzo, *Anticancer Res.*, 1994, **14**, 1059; P. Van de Velde, F. Nique, F. Bouchoux, J. Brémaud, M. C. Hameau, D. Lucas, C. Moratille, S. Viet, D. Philibert and G. Teutsch, *J. Steroid Biochem. Molec. Biol.*, 1994, **48**, 187.
14 B. Salles, C. Provot, P. Calsou, I. Hennebelle, I. Gosset and G. J. Fournié, *Anal. Biochem.*, 1995, **232**, 37.
15 C. Provot, unpublished work.

Received, 29th January 1996; Com. 6/00669H