Synthesis and Structure–Activity Relationships of the First Ferrocenyl-Aryl-Hydantoin Derivatives of the Nonsteroidal Antiandrogen Nilutamide

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Received October 7, 2007

We present here the first synthesis of organometallic complexes derived from the nonsteroidal antiandrogen nilutamide, bearing a ferrocenyl substituent at position N(1) or at C(5) of the hydantoin ring; for comparison, we also describe the C(5) *p*-anisyl organic analogue. All of these complexes retain a modest affinity for the androgen receptor. The N-substituted complexes show a weak or moderate antiproliferative effect (IC_{50} around 68 μM) on hormone-dependent and -independent prostate cancer cells, while the C(5)-substituted compounds exhibit toxicity levels 10 times higher (IC_{50} around 5.4 μM). This strong antiproliferative effect is probably due to a structural effect linked to the aromatic character of the ferrocene rather than to its organometallic feature. In addition, it seems connected to a cytotoxic effect rather than an antihormonal one. These results open the way toward a new family of molecules that are active against both hormone-dependent and hormone-independent prostate cancer cells.

1. Introduction

Prostate cancer is the most prevalent cancer and the third leading cause of cancer death in American men.[1] The natural steroid androgens, namely testosterone, the circulating hormone, and dihydrotestosterone (DHT), its active metabolite, are responsible for the development and maintenance of normal prostatic cells. However, they can also promote the malignant growth of the prostate gland, and it is known that most of the prostate cancers are androgen-dependent.[2–4] The mechanism of action of the androgens involves an interaction with a specific androgen receptor (AR[a]), and this receptor is postulated to play a crucial role in the development of prostate cancer.[5] The current treatment for prostate cancer includes a combination of surgery, radiation, and chemotherapy.[6] Clinical trials have been conducted with chemotherapeutic agents, such as docetaxel, mitoxantrone, and doxorubicin, in men with hormone refractory prostate cancer; unfortunately, these agents have limited efficacy and significant adverse side effects.[7] The therapeutic agents also include nonsteroidal antiandrogens such as nilutamide,[8,9] flutamide,[6,10–12] and bicalutamide[13] (Chart 1). However, after a period within 1−3 years, most patients relapse with advanced metastatic disease. The cancer usually becomes hormone refractory possibly because of mutations of the androgen receptor,[14] and the prognosis for these advanced metastatic prostate cancers is poor. Therefore, there is a crucial need to develop novel therapeutic agents effective on both hormone-dependent and -independent prostate cancer cells. Recently, novel candidates arising from artemisine,[15] curcumin,[16] and salicylhydrazide[17] have been published.

We have previously shown that the incorporation of a ferrocenyl unit into nonsteroidal antiestrogens such as OH-tamoxifen can lead to ferrocenyl complexes showing a strong antiproliferative effect on both hormone-dependent and -independent breast cancer cells.[18,19] However, the presence of a ferrocenyl unit is not always associated with a cytotoxic effect.[20] The IC_{50} values found for the ferrocenyl complexes covered a wide range of concentrations varying from 0.5 μM for the most active ferrocenyl-diphenol-ethene derivatives[19] to 160 μM, the value found for the ferrocene unit alone, or even higher for some ferrocenyl-estradiol derivatives.[20] This prompted us to study the effect of the introduction of a cytotoxic metal moiety into a nonsteroidal antiandrogen such as nilutamide—a topic that has not been previously investigated. We postulated that the antiandrogenic effect of nilutamide could be preserved, while the addition of an intrinsic cytotoxic function of the metal complex could lead to molecules active on both hormone-dependent and -independent prostate cancers. It has already been demonstrated that when a hydroxyalkyl or cyanoalkyl group was anchored to the NH of nilutamide it increased the affinity of the molecules for the androgen receptor, as shown with the two compounds from the former pharmaceutical company Roussel-Uclaf: 4-[4,4-dimethyl-2,5-dioxo-3-(4-hydroxybutyl)-1-imidazolidinyl]-2-trifluoromethylbenzonitrile (RU 58841)[21] and 3-[4-cyano-3-(trifluoromethyl)phenyl]-5,5-dimethyl-2,4-dioxo-1-imidazolidineacetonitrile (RU 58642)[22] (Chart 1). These molecules also bear a cyano group instead of a nitro substituent on the aromatic ring. The replacement of the nitro group prevents its reduction in vivo, and was also found to give better results for bicalutamide which contains a cyano group.[2] Therefore, we started our study by linking the ferrocenyl unit to the free NH function of nilutamide derivatives, **1–4** (Chart 2). The second approach involved the introduction of the ferrocenyl moiety at C(5) of the hydantoin ring. By doing so, we obtained **5** which is a disubstituted C(5) derivative bearing a 4-cyano-3-trifluoromethylphenyl moiety instead of a simple hydrogen. In addition, molecule **6**, a purely organic molecule related to **5**, but with a *p*-anisyl group instead of ferrocenyl, was also prepared.

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[a] Abbreviations; AR, androgen receptor; DHT, dihydrotestosterone; RBA, relative binding affinity.

10.1021/jm701264d CCC: $40.75
Published on Web 02/28/2008

Chart 1. Natural Steroidal Androgens and Representative Examples of Nonsteroidal Antiandrogens

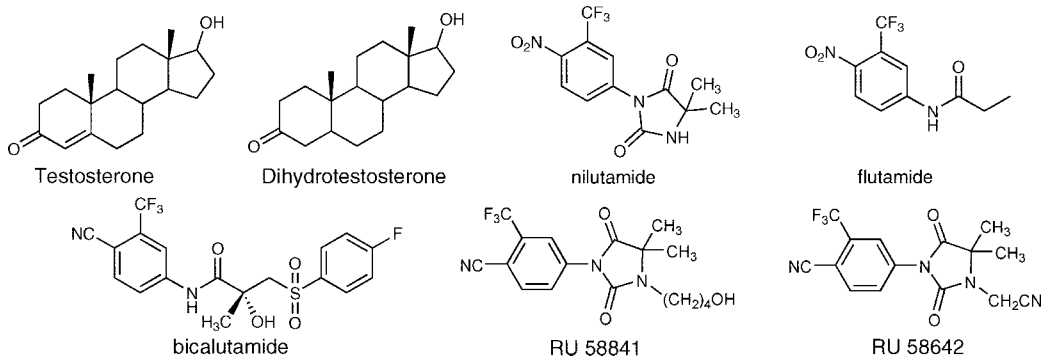
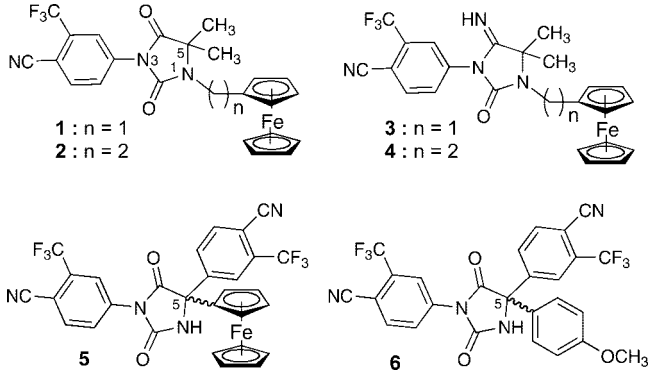
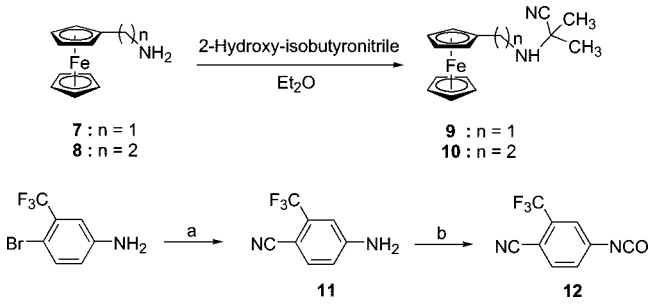


Chart 2. Newly Synthesized Molecules



Scheme 1. Synthesis of the Isocyanatobenzene **12**[a]



[a] Key: (a) (i) CuCN, DMF, (ii) aq NaCN, 92% yield; (b) $COCl_2$, toluene, AcOEt, 98%.

2. Results and Discussion

2.1. Synthesis. Synthesis of the N-Substituted Nilutamide Derivatives 1–4. The reaction of the amine **7**, derived from ferrocenecarboxaldehyde,[23] with 2-hydroxyisobutyronitrile led to the formation of ferrocenyl derivative **9** in 25% yield. Similarly, the reaction of amine **8**, obtained from the reduction of cyanomethylferrocene with $LiAlH_4$, delivered the ferrocenyl derivative **10** in 82% yield. The isocyanatobenzene, **12**, was obtained in two steps from 4-bromo-3-trifluoromethylaniline (Scheme 1).[24] The first step consisted of the aromatic nucleophilic displacement of bromide from 4-bromo-3-trifluoromethyl-aniline, by using copper cyanide, leading to the formation of 4-cyano-3-trifluoromethylaniline, **11**, in 92% yield.[25] The aniline derivative was then converted in excellent yield (98%) into the isocyanatobenzene **12** by treatment with phosgene.

The reaction of the isocyanatobenzene, **12**, with **9** and **10** furnished the imines **3** and **4**, respectively. Finally, these imines were hydrolyzed with HCl leading to the formation of the *N*-ferrocenyl hydantoins **1** and **2** in 80% and 90% yield, respectively (Scheme 2).

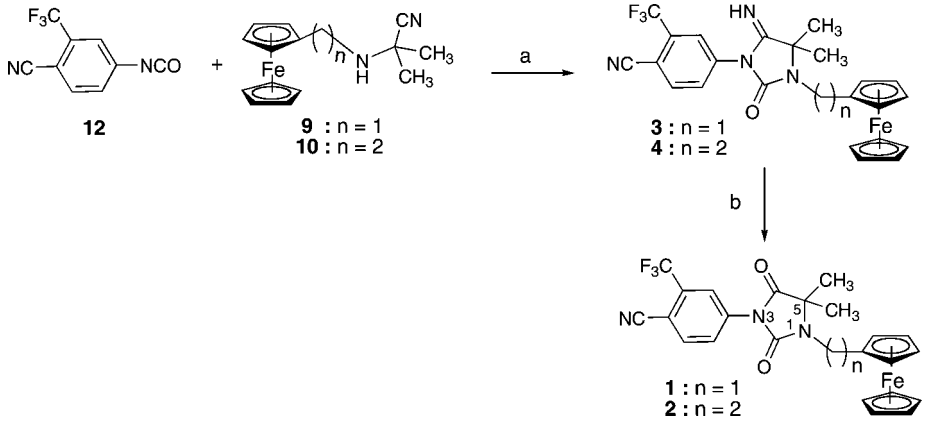
X-ray Crystal Structures of 1 and 2. The X-ray crystal structures of **1** and **2** are shown in Figures 1 and 2, respectively, and selected geometric data are also listed. There are evident similarities between the two structures which differ only by a single methylene unit. The structural parameters for both ferrocenyl substituents are within the normal ranges, and the iron atom is sandwiched almost perfectly centrally between the two cyclopentadienyl rings. However, the hydantoin and phenyl rings deviate markedly from coplanarity: in **1** and **2**, these interplanar angles are 43.8(1)° and 35.8(1)°, respectively. As shown in the space-filling representations depicted in Figure 3, the additional methylene unit in **2** places the ferrocenyl group further from the gem-dimethyls at C(5) in the hydantoin ring, thus allowing the organometallic sandwich fragment a greater degree of rotational flexibility. The interplanar angles between the hydantoin ring and the nearer cyclopentadienyl ring ($C_{17}-C_{21}$) are 68.8(1)° for **1** and 61.5(1)° for **2**.

Synthesis of C(5)-Disubstituted Nilutamide Derivatives 5 and 6. 5-Ferrocenyl-2,4-imidazolidinedione **13**, prepared from ferrocenecarboxaldehyde,[26] was allowed to react with 4-fluoro-2-trifluoromethylbenzonitrile in the presence of potassium carbonate (Scheme 3). However, not only did the substitution occur on the NH situated between the two carbonyl groups, as in nilutamide, but also at the 5-position of the hydantoin ring, already bearing the ferrocenyl moiety. The ferrocenyl hydantoin **5** was obtained in 40% yield, even though the steric hindrance at the 5-position is much greater for this ferrocenyl analogue than for nilutamide bearing two methyl groups.

The somewhat surprising disubstitution at the 5-position of the hydantoin ring in **5** prompted us to synthesize an organic analogue possessing a degree of steric hindrance similar to that caused by the ferrocenyl unit in **5**; this was done to get a realistic comparison of the steric requirements of the organometallic and organic compounds. Hence, the ferrocenyl moiety was replaced by a *p*-methoxy aromatic ring by mixing an aqueous solution of potassium cyanide and ammonium carbonate with p-anisaldehyde, thus forming the hydantoin **14**[27] (Scheme 4). As described for **5**, the hydantoin **14** was subsequently disubstituted by treatment with 4-fluoro-2-trifluoromethylbenzonitrile in the presence of potassium carbonate to form the hydantoin **6**.

2.2. Biochemical Studies. The biological properties of compounds **1–6** were tested and compared with the results obtained with the nonsteroidal antiandrogens nilutamide and bicalutamide. The relative binding affinity (RBA) values for the compounds were assayed in a standard competitive radioligand assay, using full length human recombinant AR and [^{3}-H]-DHT as a tracer. The RBA values obtained for the compounds are reported in Table 1.

Scheme 2. Synthesis of the *N*-Ferrocenyl Hydantoins **1** and **2**[a]



[a] Key: (a) 1,2-dichloroethane, NEt_3, 0 °C; (b) 2 N HCl, MeOH, reflux.

All of these compounds show an affinity for AR, as they are able to displace the binding of the [^{3}-H]-DHT to the receptor, but the RBA values found are low, i.e. less than 1%, for both the ferrocenyl complexes and nilutamide and bicalutamide. The value found for nilutamide is in accordance with the value found in the literature.[28] It is interesting to note that compounds **1** and **2** have RBA values for the AR that are distinctly higher than those of nilutamide (6 and 3 times higher, respectively) but comparable to that of bicalutamide, while the values obtained for compounds **5** and **6** are lower, by a factor of 2. The absence of hydrogen bonding between the nonsteroidal antiandrogens and amino acids T877 and N705 of the receptor has been suggested to explain their weak affinity for the receptor.[2] The same authors note that the B ring of the bicalutamide is not aligned with the mean plane of the molecule, but instead is positioned almost at a right angle. This situation is similar to that found in complexes **1** and **2** where, as noted above, the ferrocenyl group is rotated markedly relative to the other rings in the molecule. It seems therefore that a well-positioned aromatic group, whether fluorophenyl or ferrocenyl, can improve the affinity of the ligand. The effect of the newly synthesized molecules was then studied on both hormone-independent (PC-3) and hormone-dependent prostate cancer cells (LNCaP), and the results obtained in the presence of 10 μM of the different complexes are displayed in Figures 4 and 5. As expected, on PC-3 cells the antiandrogen nilutamide, whose action is supposed to be mediated by the AR, has no effect on these cells without AR. Complexes **3** and **4** show almost no antiproliferative effect (less than 10%; Table 1), bicalutamide has a weak antiproliferative effect (15%), the two N-substituted ferrocenyl complexes **1** and **2** have a significant antiproliferative effect (37−34% respectively), and the two bulky C(5)-subsituted compounds, i.e., the ferrocenyl complex **5** and the organic compound **6**, display similar, strong antiproliferative effects (84 and 76%, respectively). The IC_{50} values were then determined on PC-3 for the four compounds having an antiproliferative effect greater than 20% (Table 1). The two N-substituted ferrocenyl complexes **1** and **2** show IC_{50} values of 68 and 67 μM, while the two most active compounds, **5** and **6**, display IC_{50} values equal to 5.4 and 5.6 μM, i.e., values 10 times lower than the ones found for **1** and **2**. An almost identical IC_{50} value (7.4 μM) was found for **5** on MDA-MB-231 cells (hormone-independent breast cancer cells). These values are lower than the value found, on MDA-MB-231 cells, for the reference drug cisplatin (12.7 μM).[29]

The results obtained on the hormone-dependent prostate cancer cells LNCaP are shown in Figure 5. As expected for these cells with androgen receptors, the natural androgen DHT

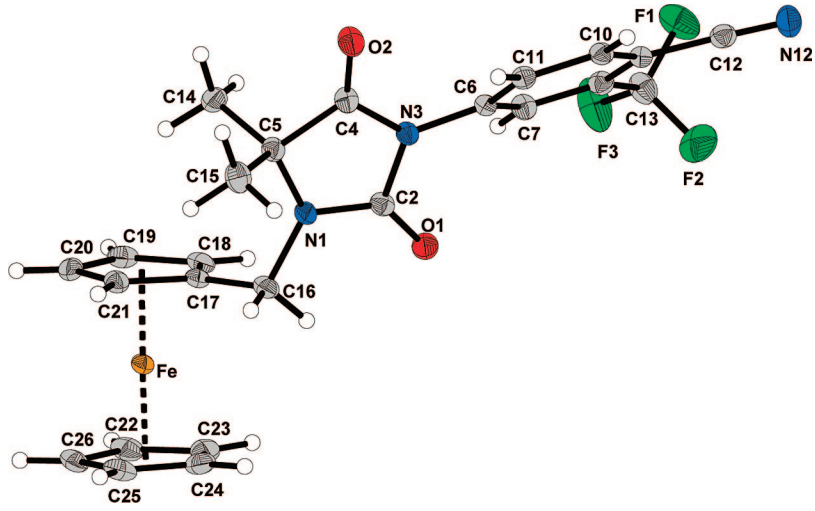


Figure 1. X-ray crystal structure of **1**. Selected bond lengths (Å) and angles (deg): Fe−Cent(1) 1.649(2); Fe−Cent(2) 1.644(1); C_{17}−C_{16} 1.497(2); C_{16}−N_1 1.4671(18); N_1−C_2 1.3467(18); C_2−N_3 1.4286(18); N_3−C_4 1.3875(18); C_4−C_5 1.5313(19); C_5−N_1 1.4696(18); N_3−C_6 1.4194(18); C_9−C_{12}, 1.446(2); C_{12}−N_{12} 1.144(2); C_8−C_{13}, 1.501(2); C_2−O_1, 1.2120(18); C_4−O_2, 1.2045(18); C_{21}−C_{17}−C_{18}, 107.63(13); C_{17}−C_{16}−N_1 112.66(12); N_1−C_2−N_3, 107.04(12); C_2−N_3−C_4 110.87(11); N_3−C_4−C_5 107.23(12); C_4−C_5−N_1 101.14(11); C_5−N_1−C_2 113.50(11).

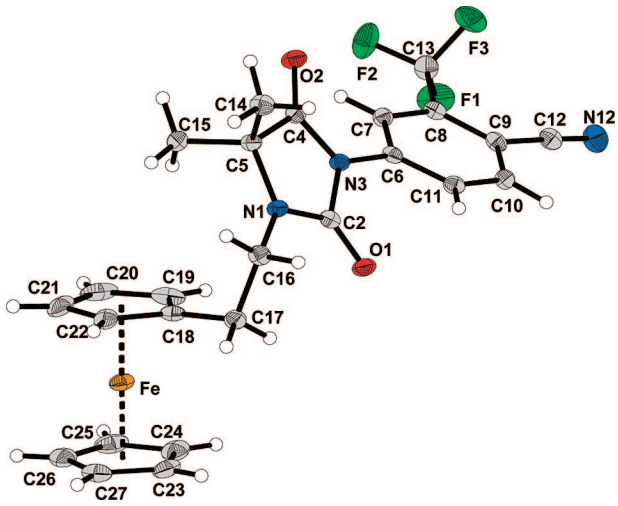


Figure 2. X-ray crystal structure of **2**. Selected bond lengths (Å) and angles (deg): Fe−Cent(1), 1.646(1); Fe−Cent(2), 1.645(1); $C_{18}-C_{17}$ 1.492(3); $C_{17}-C_{16}$ 1.528(3); $C_{16}-N_1$ 1.468(2); N_1-C_2 1.334(2); C_2-N_3 1.431(2); N_3-C_4 1.388(2); C_4-C_5 1.528(2); C_5-N_1 1.468(2); N_3-C_6 1.417(2); C_9-C_{12}, 1.444(2); $C_{12}-N_{12}$ 1.143(2); C_8-C_{13}, 1.503(2); C_2-O_1, 1.211(2); C_4-O_2, 1.206(2); $C_{19}-C_{18}-C_{22}$, 106.83(17); $C_{18}-C_{17}-C_{16}$ 112.66(15); $C_{17}-C_{16}-N_1$ 113.32(15); $N_1-C_2-N_3$, 107.29(14); $C_2-N_3-C_4$ 110.45(14); $N_3-C_4-C_5$ 107.23(14); $C_4-C_5-N_1$ 101.21(13); $C_5-N_1-C_2$ 113.57(14).

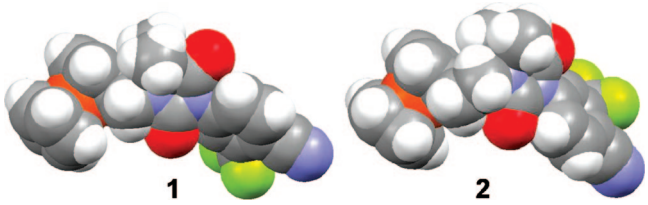
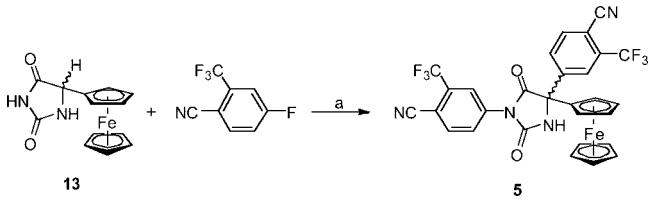


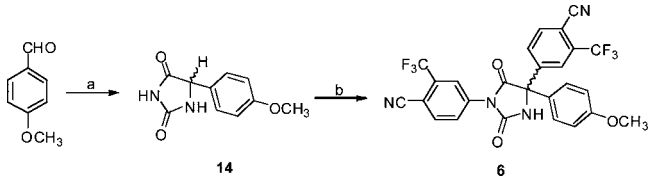
Figure 3. Space-filling views of the ferrocenyl complexes **1** and **2**.

Scheme 3. Synthesis of the C(5)-Ferrocenyl Derivative **5**[a]



[a] Key: (a) K_2CO_3, DMF, 80 °C, 40% yield.

Scheme 4. Synthesis of the C(5)-Diphenyl Hydantoin Derivative **6**[a]



[a] Key: (a) KCN, $(NH_4)_2CO_3$, H_2O/EtOH, 60 °C, 42% yield; (b) 4-fluoro-2-trifluoromethylbenzonitrile, K_2CO_3, DMF, 80 °C, 30% yield.

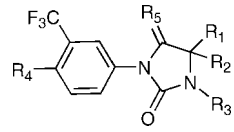
displays a proliferative effect. Bicalutamide shows a rather weak antiproliferative effect, nilutamide has an unexpected but clear and reproducible proliferative effect, while **5** and **6** have an almost identical strong antiproliferative effect. The addition of DHT reverses the antiproliferative effect of bicalutamide, but not the effect observed with **5** and **6**. This could be explained by the fact that the modest effect observed with bicalutamide is an antihormonal effect mediated by androgen receptors while the effect of **5** and **6** is connected to an intrinsic cytoxicity of the molecules.

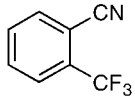
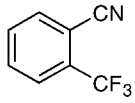
Finally, the values found for the lipophilicity of the new molecules are higher than the log $P_{o/w}$ values of bicalutamide and nilutamide. This is expected as they all possess phenyl or ferrocenyl substituents which are known to be lipophilic.

3. Discussion

These results raise a number of points, regarding both the newly synthesized products and the nonsteroidal antiandrogens, which are good candidates for treatment of prostate cancer at an early stage (bicalutamide) or postcastration late-stage (nilutamide). We were unable to find literature data on testing of these compounds on cell lines. This may be because the compounds reached the market at a time before cell-line testing was as widespread as it is today. It should also be noted that, unlike breast cancer, for which several hormone-dependent and hormone-independent cell lines are available, the choice of hormone-dependent human prostate cancer cell lines is very restricted and essentially limited to LNCaP cells. However, it is known that the androgen receptor expressed by these cells is a mutated one that has been described as having a modified response to antiandrogens.[30] It is thus plausible that the proliferative effect observed may be linked to this mutation. In terms of the newly synthesized products, it can be noted that the attachment to N(1) of a ferrocenyl substituent, via an aliphatic chain with one or two links, increases toxicity relative to that of nilutamide essentially only in the case of the derivatives of **1** and **2**, and not of their precursors **3** and **4**. The two most cytotoxic compounds are **5** and **6** which have bulky lipophilic substituents attached directly onto C_5 of the hydantoin ring. The IC_{50} values of these compounds are of the same order of magnitude as those found for other ferrocenyl complexes, in particular certain complexes with an sp^3 carbon.[31] However, the toxicity of **5** does not appear to be linked to the organometallic feature of the ferrocenyl substituent or to the presence of an iron atom, since its organic analogue, **6**, shows the same antiproliferative effect. What the two substituents have in common is their aromaticity, which increases their lipophilicity compared to the parent compound nilutamide (3.2 for nilutamide, 6.5 and 5.7 for **5** and **6**; see Table 1). Furthermore, it has been previously shown in a QSAR (quantitative structure–activity relationship study) of phenolic compounds that an increase in the lipophilicity correlates with an increase in their cytotoxicity.[32] Hence, this may be the source of the antiproliferative effect observed with our molecules. It should also be noted that there is a certain analogy between the chemical structure of compounds **5** and **6** and that of hydantoins that have recently been reported which are substituted at the C_5 position by aromatics or by electron-rich substituents.[33–35] The aromatic hydantoins show an affinity for the cannabinoid receptor,[34,35] while electron-rich substituents induce activity for the calcium channels, at the site of verapamil.[33] Moreover, it has been shown that certain ligands of the cannabinoid receptor can induce apoptosis of the LNCaP and PC-3 prostate cancer cells[36,37] and that verapamil is capable of inhibiting proliferation of LNCaP cells via an interaction at the level of the potassium channels.[38] It would therefore be

Table 1. RBA for AR, Antiproliferative Effect, and IC_{50} Values on PC-3 (Hormone-Independent Prostate Cancer Cells) and log $P_{o/w}$ of the Compounds



compound	R_1	R_2	R_3	R_4	R_5	RBA (%) on AR[a)]	Antiproliferative effect (%) on PC-3 at 10 μM	IC_{50} (μM) on PC-3 [b)]	$logP_{o/w}$
bicalutamide						0.29 ± 0.02	15	-	3.4
nilutamide	CH_3	CH_3	H	NO_2	O	0.15 ± 0.03	11	-	3.2
1	CH_3	CH_3	CH_2-Fc [c)]	CN	O	0.52 ± 0.13	37	68 ± 28	5.2
2	CH_3	CH_3	$(CH_2)_2$-Fc [c)]	CN	O	0.22 ± 0.05	34	67 ± 2	5.6
3	CH_3	CH_3	CH_2-Fc [c)]	CN	NH	0.070 ± 0.004	4	-	4.7
4	CH_3	CH_3	$(CH_2)_2$-Fc [c)]	CN	NH	0.09 ± 0.03	9	-	5.0
5		Fc [c)]	H	CN	O	0.041± 0.02	84	5.4 ± 0.5	6.5
6		p-anisyl	H	CN	O	0.041± 0.005	76	5.6 ± 0.4	5.7

[a] Mean of two experiments ± range. [b] IC_{50} values were determined only for compounds having an antiproliferative effect, on PC-3 at 10 μM, higher than 20%. [c] Fc = ferrocenyl (η^5-C_5H_4-Fe-C_5H_5).

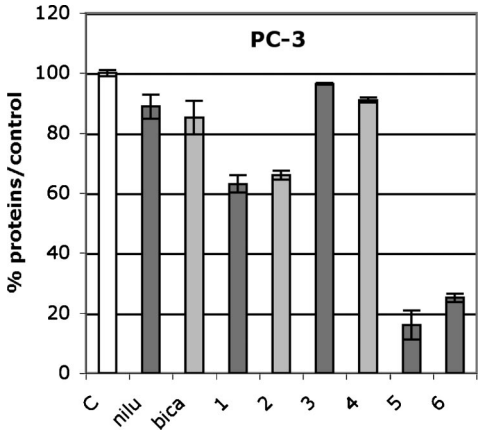


Figure 4. Effect of 10 μM of the nonsteroidal antiandrogens nilutamide (nilu) and bicalutamide (bica) and of the new compounds (**1**−**6**) on the growth of hormone-independent prostate cancer cells PC-3, after 5 days of culture. Nontreated PC-3 cells are used as the control (C). Mean of two separate experiments (six measurements for each one) ± range.

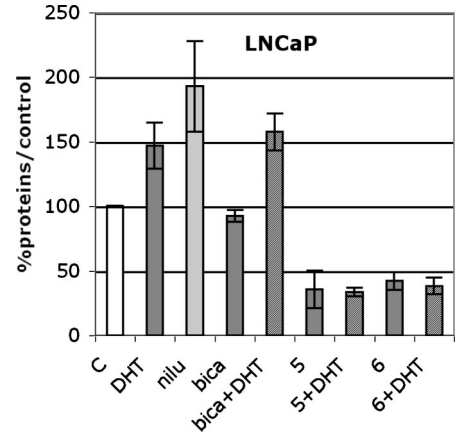


Figure 5. Effect of 10 nM of the natural androgen DHT and of 10 μM of the nonsteroidal antiandrogens nilutamide (nilu), bicalutamide (bica) and of **5** and **6** in the absence or presence of 10 nM DHT on the growth of hormone-dependent prostate cancer cells LNCaP, after 5 days of culture. Nontreated LNCaP cells are used as the control (C). Mean of two separate experiments (six measurements for each one) ± range.

worthwhile to investigate further in order to establish whether our compounds act via one or other of these pathways.

Conclusion

A number of reasons have been put forward to justify the medical use of metallocenes, of which ferrocene is the archetype, attached to biological nanovectors.[39–41] These moieties are small, rigid, lipophilic, and easily able to cross cell membranes even after substitution. They are also similar in shape to an aromatic nucleus, even if their sandwich structure makes them somewhat thicker. Finally, the ferrocenyl compounds sometimes show an unusual redox activity in terms of the target. An illustration of these different behaviors was provided by our study of their antiproliferative effects on both hormone-dependent and non-hormone-dependent breast cancer cells. The novel molecules **5** and **6**, the most effective in the current study, show an unusual behavior. Their activity does not occur via the androgen receptor, even in the case of LNCaP cells. The introduction of a ferrocenyl group produces the same effect as a purely organic aromatic moiety. Structures **5** and **6** may act via recognition by other receptors. Of these, the cannabinoid receptors suggest an interesting hypothesis, since a higher level of these receptors is found in the cancerous prostate relative to the healthy organ,[37] and structures somewhat analogous to our molecules are active by this route. Further studies are underway on this series of molecules to test this particular mechanistic hypothesis. In any case, the activity of these structures merits further attention in itself.

4. Experimental Section

4.1. General Comments. All reactions were carried out under an atmosphere of argon. Diethyl ether, ethyl acetate, and toluene were distilled from sodium/benzophenone. All other chemical reagents and solvents were used as purchased without further purification. Column flash chromatography was performed on silica gel Merck 60 (40–63 μm). Melting points were measured with a Kofler device. Infrared spectra were recorded on an IR-FT BOMEM Michelson-100 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz Bruker or a 600 MHz Varian spectrometer; assignments were made by standard 2-D HSQC and HMBC techniques. Elemental analyses were performed by the Service de Microanalyse I.C.S.N., Gif sur Yvette, France. Analytical HPLC was performed on a C18 Kromasil column 10 μm, $L = 25$ cm, $D = 4.6$ mm, eluent: acetonitrile/water 80:20, flow rate = 1 mL/min, $\lambda = 254$ nm.

4.2. Synthesis. Compounds **7**, **8**,[42] and **11**[25] were prepared following the literature procedures.

4-(4,4-Dimethyl-2,5-dioxo-3-ferrocenylmethyl-1-imidazolidinyl)-2-trifluoromethylbenzonitrile (1). Aqueous HCl solution (2 N, 3.2 mL) was added to a solution of imine **3** (0.390 g, 0.79 mmol) in methanol (20 mL). The reaction mixture was heated under reflux for 2 h. The solution was then cooled to room temperature and was poured into cold water (100 mL). The mixture was extracted with ethyl acetate, and the organic phase was dried on magnesium sulfate, filtered, and evaporated to give **1** as a yellow-orange solid (0.340 g, 80% yield): mp 173–174 °C; IR (KBr) 2230 (CN), 1777 and 1716 (2 CO) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (s, 6H, 2 CH_3), 4.17 (t, 2H, C_5H_4, $J = 1.8$ Hz), 4.19 (s, 5H, C_5H_5), 4.32 (t, 2H, C_5H_4, $J = 1.8$ Hz), 4.41 (s, 2H, CH_2N), 7.89 (d, 1H, H10, $J = 8.7$ Hz), 7.98 (dd, 1H, H11, $J = 8.7$ Hz, $J = 1.8$ Hz), 8.14 (d, 1H, H7, $J = 1.8$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.7 (2 CH_3), 39.8 (CH_2N), 61.9 ($C(CH_3)_2$), 68.8 (C_5H_4), 68.9 (C_5H_5), 70.0 (C_5H_4), 82.7 (C_5H_4, C_{ip}), 115.2 (CN), 123.1 (C7), 128.0 (C11), 134.0 (q, C8), 135.3 (C10), 136.5 (C6), 152.7 (CO), 174.8 (CO); EI-MS *m/z* 495 [M$^{+\bullet}$]. Anal. ($C_{24}H_{20}F_3FeN_3O_2$) C, H, N.

4-(4,4-Dimethyl-2,5-dioxo-3-ferrocenylethyl-1-imidazolidinyl)-2-trifluoromethylbenzonitrile (2). Aqueous HCl solution (2 N, 3.2 mL) was added to a solution of imine **4** (0.620 g, 1.22 mmol) in methanol (40 mL). The reaction mixture was heated under reflux for 1 h. The solution was then cooled to room temperature and was poured into cold water (100 mL). The mixture was extracted with ethyl acetate, and the organic phase was dried on magnesium sulfate, filtered, and evaporated giving **2** as a yellow-orange solid (0.60 g, 89% yield): mp 140–142 °C; IR (KBr) 2230 (CN), 1777 and 1720 (2 CO) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (s, 6H, 2 CH_3), 2.79 (m, 2H, CH_2), 3.44 (m, 2H, CH_2N); 4.11 (s, 4H, C_5H_4), 4.15 (s, 5H, C_5H_5), 7.92 (d, 1H, H10, $J = 8.4$ Hz), 8.02 (dd, 1H, H11, $J = 8.4$ Hz, $J = 1.8$ Hz), 8.17 (d, 1H, H7, $J = 1.8$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.5 (2 CH_3), 29.3 (CH_2), 41.9 (CH_2N), 62.0 ($C(CH_3)_2$), 67.9, 68.3 (C_5H_4), 68.8 (C_5H_5), 84.4 (C_5H_4, C_{ip}), 108.3 (C9), 115.1 (CN), 122.0 (q, CF_3), 123.0 (C7), 127.9 (C11), 134.0 (q, C8), 135.3 (C10), 136.6 (C6), 152.8 (CO), 174.7 (CO); EI-MS *m/z* 509 [M$^{+\bullet}$]. Anal. ($C_{25}H_{22}F_3FeN_3O_2$) C, H, N.

4-(4,4-Dimethyl-3-ferrocenylmethyl-5-imino-2-oxo-1-imidazolidinyl)-2-trifluoromethylbenzonitrile (3). A solution of isocyanate **12** (1.35 g, 6.35 mmol) in 1,2-dichloroethane (50 mL) was added dropwise at 0 °C to a solution of **9** (1.79 g, 6.35 mmol) in 1,2-dichloroethane (30 mL) and triethylamine (0.6 mL). The reaction mixture was stirred at 0 °C for 2 h. The solution was heated to room temperature; the solvent was then evaporated under reduced pressure. The crude residue was purified by column chromatography (SiO_2; diethyl ether), and **3** was obtained as a yellow-orange solid (1.16 g, 34% yield): mp 220 °C; IR (KBr) 3281 (NH) 2229 (CN) 1723 (CO) 1661 (imine) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (s, 6H, 2 CH_3), 4.15 (t, 2H, C_5H_4, $J = 1.8$ Hz), 4.18 (s, 5H, C_5H_5), 4.31 (t, 2H, C_5H_4, $J = 1.8$ Hz), 4.38 (s, 2H, CH_2), 7.42 (s, 1H, *N*═H), 7.92 (d, 1H, H10), 8.08 (s, 1H, H11), 8.24 (d, 1H, H7); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.6 (2 CH_3), 39.7 (CH_2), 61.1 ($C(CH_3)_2$), 68.6 (C_5H_4), 68.9 (C_5H_5), 69.9 (C_5H_4), 83.3 (C_5H_4,C_{ip}), 124.3 (C7), 129.1 (C11), 135.0 (C10), 153.2 (CO), 168.3 (*C*═NH); EI-MS *m/z* 494 [M$^{+\bullet}$]. Anal. ($C_{24}H_{21}F_3FeN_4O$) H, N. C: calcd 58.32, found 57.79.

4-(4,4-Dimethyl-3-ferrocenylethyl-5-imino-2-oxo-1-imidazolidinyl)-2-trifluoromethylbenzonitrile (4). A solution of isocyanate **12** (0.716 g, 3.38 mmol) in 1,2-dichloroethane (50 mL) was added dropwise at 0 °C to a solution of **10** (1.00 g, 3.38 mmol) in 1,2-dichloroethane (30 mL) and triethylamine (0.3 mL), and the reaction mixture was stirred at 0 °C for 2 h. The solution was allowed to warm to room temperature, and the solvent was then evaporated under reduced pressure. The crude residue was purified by column chromatography (SiO_2; diethyl ether), and **4** was obtained as a yellow-orange solid (1.10 g, 59% yield). mp 210 °C; IR (KBr) 3283 (NH), 2234 (CN), 1725 (CO), 1662 (imine) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (s, 6H, 2 CH_3), 2.76 (m, 2H, CH_2), 3.40 (m, 2H, CH_2N), 4.10 (s, 4H, C_5H_4), 4.15 (s, 5H, C_5H_5), 7.42 (s, 1H, *N*═H), 7.92 (d, 1H, H10), 8.08 (d, 1H, H11), 8.24 (s, 1H, H7); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.4 (2 CH_3), 29.5 (CH_2), 41.9 (CH_2N), 61.4 ($C(CH_3)_2$), 67.9, 68.4 (C_5H_4), 68.8 (C_5H_5), 84.7 (C_5H_4, C_{ip}), 124.4 (C7), 129.3 (C11), 135.3 (C10), 153.4 (CO), 168.3 (═NH); EI-MS *m/z* 508 [M$^{+\bullet}$]. Anal. ($C_{25}H_{23}F_3FeN_4O$) C, H, N.

3-(4-Cyano-3-trifluoromethylphenyl)-5-(ferrocenyl)-5-(4-cyano-3-trifluoromethylphenyl)imidazolidine-2,4-dione (5). Compound **13** (1.13 g, 3.96 mmol) and potassium carbonate (0.55 g, 3.96 mmol) were added to a solution of 4-fluoro-2-trifluoromethylbenzonitrile (0.500 g, 2.64 mmol) in anhydrous DMF (30 mL). The reaction mixture was stirred at 80 °C for 4 h. Ethyl acetate was then added to the solution, which was filtered on Celite. The filtrate was evaporated to give a crude product which was purified by chromatography (SiO_2; ethyl acetate/petroleum ether 2:3 → 1:2). Compound **5** was obtained as a yellow orange solid (1.06 g, 40% yield): mp 200–202 °C; IR (KBr) 3309 (NH), 2238 (CN) 1792 and 1739 (2 CO) cm^{-1}; ^{1}H NMR (600 MHz, acetone-d_6) δ 4.24 (s, 5H, C_5H_5), 4.40 (m, 1H), 4.42 (m, 1H), 4.43 (m, 1H), 4.77 (m,1H) (C_5H_4), 8.11 (d, 1H, $J = 8.5$ Hz, H16), 8.15 (d, 1H $J = 8.5$ Hz, H17), 8.16 (s, 1H, H13), 8.29 (d, 1H, $J = 8.5$ Hz, H10), 8.32 (d, 1H, $J = 8.5$ Hz, H11), 8.34 (s, 1H, H7), 9.19 (s, 1H, NH); ^{13}C NMR (150 MHz, acetone-d_6) δ 66.74 (C_5H_4), 67.77 (C5), 67.85 (C_5H_4), 69.73 (C_5H_4), 69.81 (C_5H_5), 70.45 (C_5H_4), 90.57 (C_5H_4, C_{ipso}), 109.00 (q, $J = 2$ Hz, C9), 110.37 (q, $J = 2$ Hz, C15), 115.67 (C9-*C*N), 115.71 (C15-*C*N), 123.32 (q, $J = 273$ Hz, C8-*C*F_3), 123.48 (q, $J = 273$ Hz, C15-*C*F_3), 124.17 (q, $J = 5$ Hz, C7), 125.39 (q, $J = 5$ Hz, C13), 129.88 (C11), 131.93 (C17), 132.57 (q, $J = 32$ Hz, C14), 133.28 (q, $J = 33$ Hz, C8), 136.30 (C16), 137.00 (C10), 137.49 (C6), 146.04 (C12), 154.38 (*C*2), 170.71 (C4); EI-MS *m/z* 622 [M$^{+\bullet}$]. Anal. ($C_{29}H_{16}F_6FeN_4O_2$) C, H, N.

3-(4-Cyano-3-trifluoromethylphenyl)-5-(4-methoxyphenyl)-5-(4-cyano-3-trifluoromethylphenyl)imidazolidine-2,4-dione (6). Compound **14** (0.310 g, 1.50 mmol), 4-fluoro-2-trifluoromethylbenzonitrile (0.600 g, 3.15 mmol), and potassium carbonate (0.430 g, 3.15 mmol) were mixed in distilled DMF (10 mL). The reaction mixture was stirred at 80 °C for 4 h before it was filtered over a short pad of Celite. The solvent was evaporated to yield a yellow oil which was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 2:3). The second fraction corresponded to the pure product as a white solid (0.245 g, 30% yield): mp degradation at 82 °C; IR (KBr) 3305 (NH) 2235 (CN) 1793 and 1738 (CO) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.83 (s, 3H, OCH_3), 6.92–6.98 (m, 2H, H20 and H22), 7.09 (s, 1H, NH), 7.18–7.23 (m, 2H, H13 and H19), 7.80–7.97 (m, 5H, H13, H16, H17, H10 and H11), 8.09 (s, 1H, H7); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.5 (OCH_3), 69.2 (C5), 109.3, 110.7 (C9 and C15), 114.6 (CN), 115.1 (C20 and C22), 120.1 (CF_3) 123.3, 125.1 (C7 and C13), 127.7 (C19 and C23), 128.3 (C7), 129.1 (C18), 131.0 (C13), 133.3, 134.1 (C8 and C14, $J = 33.2$ Hz), 135.2 (C10), 135.4 (C6), 135.5 (C16), 143.7 (C12), 153.5 (CO), 160.6 (C18), 170.1 (CO); EI-MS *m/z* 544 [M$^{+\bullet}$]. Anal. ($C_{26}H_{14}F_6N_4O_3$) C, H, N.

2-Cyano-2-[*N*-(ferrocenylmethyl)amino]propane (9). The amine **7** (0.500 g, 2.33 mmol) was dissolved in diethyl ether (12 mL). This solution was added slowly to 2-hydroxyisobutyronitrile (2.7 mL, 31.5 mmol). The mixture was stirred for 3 h at room

temperature, water was added, the mixture was extracted with diethyl ether, and the organic phase was dried on magnesium sulfate, filtered, and evaporated to give an orange oil. The product was purified by filtration on silica gel (diethyl ether/petroleum ether 1:1), yielding **9** as an orange solid (0.190 g, 25% yield): mp 62 °C; IR (KBr) 3313 (NH), 2219 (CN) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.51 (s, 6H, 2 CH_3), 3.60 (s, 2H, CH_2NH), 4.14 (t, 2H, C_5H_4, J = 1.8 Hz), 4.16 (s, 5H, C_5H_5), 4.21 (t, 2H, C_5H_4, J = 1.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.6 (2 CH_3), 44.5 (CH_2NH), 51.8 ($C(CH_3)_2$), 68.1, 68.2 (C_5H_4), 68.6 (C_5H_5), 86.0 ($C_5H_{4\ ipso}$), 123.0 (CN); EI-MS m/z 282 [$M^{+\bullet}$]. Anal. ($C_{15}H_{18}FeN_2$) C, H, N.

2-Cyano-2-[*N*-(ferrocenylethyl)amino]propane (10). Amine **8** (1.00 g, 4.36 mmol) was dissolved in diethyl ether (18 mL), and this solution was added slowly to 2-hydroxyisobutyronitrile (2.5 mL, 27.5 mmol). The reaction mixture was stirred for 3 h at room temperature, water was added, and the mixture was extracted with diethyl ether. The organic phase was dried on magnesium sulfate, filtered, and evaporated to give an orange oil. The product was purified by filtration on silica gel (diethyl ether/petroleum ether 1:1), yielding **10** as an orange oil (1.22 g, 82% yield). This oil was crystallized from pentane to give an orange solid: mp 74–76 °C; IR (KBr) 3314 (NH) 2218 (CN) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 6H, 2 CH_3), 2.57 (t, 2H, CH_2, J = 7.2 Hz), 2.87 (t, 2H, CH_2NH, J = 7.2 Hz), 4.08 (t, 2H, C_5H_4), 4.11 (s, 5H, C_5H_5), 4.13 (t, 2H, C_5H_4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.5 (2 CH_3), 30.2 (CH_2), 46.0 (CH_2NH), 51.5 ($C(CH_3)_2$), 67.7, 68.4 (C_5H_4), 68.7 (C_5H_5), 85.7 (C_5H_4, C_{ipso}), 122.8 (CN); EI-MS m/z 296 [$M^{+\bullet}$]. Anal. ($C_{16}H_{20}FeN_2$) C, H. N: calcd 9.46, found 8.83.

4-Cyano-3-trifluoromethyl-isocyanatobenzene (12). [24] A solution of 4-cyano-3-trifluoromethylaniline **11** (2.00 g, 10.8 mmol) in distilled ethyl acetate (15 mL) was added dropwise at 0 °C to a solution of 20% (m/v) phosgene in toluene (7 mL, 13.0 mmol). The reaction vessel was set up like a distillation apparatus and was equipped to trap the HCl formed. After being stirred at 0 °C for 30 min, the reaction mixture was allowed to warm to room temperature and then was heated under reflux. A part of the solvent was distilled and was replaced by distilled toluene (20 mL) until the temperature reached 110 °C. Reflux was continued until HCl evolution subsided. A precipitate was removed by filtration, and the filtrate was evaporated under vacuum. The product **12** was obtained as an orange oil (2.24 g, 98% yield). This oil was directly used for the next step: IR (CH_2Cl_2) 2268 (NCO) 2233 (CN) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.39 (dd, 1H, H6, J = 8.3 Hz, J = 2.2 Hz), 7.50 (d, 1H, H2, J = 2.2 Hz), 7.82 (d, 1H, H5, J = 8.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 107.1 (C4), 114.9 (CN), 121.7 (q, CF_3, J = 265.3 Hz), 123.4 (*C*2), 126.3 (NCO), 128.4 (C6), 134.2 (C3), 136.3 (C5), 138.7 (C1).

5-(Ferrocenyl)imidazolidine-2,4-dione (13). [26,43] A solution of formylferrocene (1.00 g, 4.67 mmol) in ethanol (25 mL) was added to 20 mL of $NaHSO_3$ aqueous solution (0.97 g). In another Schlenk tube, potassium cyanide (1.37 g, 20 mmol) and ammonium carbonate (4.04 g, 42 mmol) were dissolved in a mixture of distilled water (27 mL) and ethanol (5 mL). The first solution was then added to the second solution. The mixture was heated for 2 h at 60 °C. The reaction mixture was cooled to room temperature, and ethanol was removed under vacuum. A yellow solid obtained was filtered and washed with diethyl ether to give the pure product (0.52 g, 39% yield): mp 252–254 °C; IR (KBr) 3407 and 3203 (2 NH), 1775 and 1724 (2 CO) cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 4.25 (s, 5H, C_5H_5), 4.28 and 4.47 (2 t, 4H, C_5H_4, J = 1.2 Hz), 5.85 (s, 1H, H5), 7.79 (s, 1H, NH), 9.57 (s, 1H, NH);. ^{13}C NMR (75 MHz, acetone-d_6) δ 56.7 (C5), 65.3, 66.5, 67.7, 69.1, 84.1 (C_5H_4), 69.0 (C_5H_5), 157.4 (*C*=O), 173.5 (*C*=O).

5-(4-Methoxyphenyl)imidazolidine-2,4-dione (14). [27] Potassium cyanide (0.800 g, 0.012 mol) and ammonium carbonate (4.80 g, 0.05 mol) were dissolved in distilled water (27 mL). A solution of *p*-anisaldehyde (0.63 mL, 0.01 mol) in absolute ethanol (20 mL) was then added dropwise at room temperature. The colorless solution was heated for 4 h at 60 °C and 2 h at 110 °C to eliminate the excess of ammonium carbonate. The reaction mixture was cooled to 0 °C before concentrated HCl was added dropwise until pH 2–3 of the solution was reached. A white solid appeared and was filtered off to give the pure product (0.86 g, 42% yield): mp 193 °C; ^1H NMR (300 MHz, acetone-d_6) δ 3.66 (s, 3H, OCH_3), 5.01 (s, 1H, CH), 6.82 (d, 2H, J = 9.0 Hz, C_6H_4), 7.22 (d, 2H, J = 8.7 Hz, C_6H_4), 7.22 (s, 1H, NH), 9.55 (s, 1H, NH); ^{13}C NMR (75 MHz, acetone-d_6) δ 55.6 (OCH_3), 62.3 (CH), 114.9, 128.8, 128.9 (C_6H_4), 157.9 (*C*=O), 160.8 (C_6H_4), 174.4 (*C*=O); CI-MS m/z 491 [MNH_4^+].

Bicalutamide. Bicalutamide was prepared following the literature procedure:[44] mp 190–193 °C; ^1H NMR (300 MHz, acetone-d_6): δ1.54 (s, 3H,), 3.68 (d, 1H, CH_2SO_2, J = 14.8 Hz), 4.07 (d, 1H, CH_2SO_2, J = 14.8 Hz), 5.52 (s, 1H, OH), 7.28 and 7.30 (d, d, 1H, 1H, C_6H_4, J = 8,8 Hz), 7.96–8.01 (m, 2H, C_6H_4), 8.02 (d, 1H, C_6H_3, J = 8,9 Hz), 8.21 (dd, 1H, C_6H_3, J = 8.9 Hz, J = 2.1 Hz), 8,41 (d, 1H, C_6H_3, J = 2.1 Hz), 9.94 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.2 (CH_3), 63.5 (CH_2SO_2), 73.2 (C(OH)). 102.0 (C_6H_3, C_{ipso}), 114.5 (CN), 115.9 and 116.2 (C_6H_4), 117.5 (C_6H_3), 121.5 (q, CF_3, J = 273.8 Hz), 122.9 (C_6H_3), 131.3 and 131,4 (C_6H_4), 134.8 (q, C_6H_3), 136.2 (C_6H_3), 137,1 (C_6H_4, C_{ipso}), 143.2 ((C_6H_3, C_{ipso}), 163.9 (C_6H_4, C_{ipso}), 173.7 (NHCO); EI-MS m/z 430 [$M^{+\bullet}$]. Anal. ($C_{18}H_{14}F_4N_2O_4S$) C, H, N.

4.3. X-ray Crystal Structure Determinations for 1 and 2. Crystal data were collected using a Bruker SMART APEX CCD area detector diffractometer and are listed in Table S1 (Supporting Information). A full sphere of reciprocal space was scanned by φ-ω scans. Pseudoempirical absorption correction based on redundant reflections was performed by the program SADABS.[45]The structures were solved by direct methods using SHELXS-97[46] and refined by full-matrix least-squares on F^2 for all data using SHELXL-97.[47] All hydrogen atoms were located in the difference Fourier map and allowed to refine freely with isotropic thermal displacement factors. Anisotropic thermal displacement parameters were used for all nonhydrogen atoms.

Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-655743 (**1**) and CCDC-655742 (**2**). Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ (Fax: +44-1223-336033; E-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

4.4. Biochemical Experiments. a. Purity of the Compounds. The purity of the compounds tested in biochemical experiments (i.e., **1**–**6** and bicalutamide) was checked by reversed-phase HPLC on a Chromasil C18 column (10 μm, L = 25 cm D = 4.6 mm) eluted with a mixture acetonitrile/water (80/20) and measurement of the absorbance at 254 nm.

b. Materials. DHT, nilutamide, and protamine sulfate was obtained from Sigma-Aldrich (France). Stock solutions (1×10^{-3} M) of the compounds to be tested were prepared in DMSO and were kept at −20 °C. Under these conditions, they are stable for at least two weeks. Serial dilutions in DMSO were prepared just prior to use. Dulbecco's modified eagle medium (DMEM) was purchased from Invitrogen. Fetal calf serum, glutamine and kanamycine were obtained from Invitrogen. Prostate and breast cancer cells LNCaP, PC3, and MDA-MB231 cells were from the American type Culture Collection (ATCC). [1,2-^{3}H]-DHT was purchased from NEN Life Science Product.

c. Determination of the RBA of the Compounds for the AR. RBA values were measured on AR PanVera (750 pmol) purchased from Invitrogen. The ARs were aliquoted in 15 μL fractions and kept in liquid nitrogen until used. For each experiment, 10 mL of buffer containing 10% glycerol, 50 mM Tris pH 7.5, 0.8 M NaCl, 2 mM DTT, and 0.1% BSA was added to one aliquot. Fractions of 200 μL of the AR solution were incubated in polypropylene tubes for 3 h 30 at 4 °C with [1,2- ^{3}H]-DHT (2×10^{-9}M , specific activity 1.6 TBq/mmol) in the presence of nine concentrations of the compounds to be tested (between 1×10^{-5} M and 6×10^{-7} M) or of non radioactive DHT (between 8×10^{-8} and 7.5×10^{-10} M). At the end of the incubation period, the fractions of [^{3}H]-DHT bound to the androgen receptor (Y values) were precipitated by addition of a 200 μl of a cold solution of

protamine sulfate (1 mg/mL in water). After a 10 min period of incubation at 4 °C, the precipitates were recovered by filtration on 25 mm circle glass microfiber filters GF/C filters using a Millipore 12 well filtration ramp. The filters were rinsed twice with cold phosphate buffer and then transferred in 20 mL plastic vials. After addition of 5 mL of scintillation liquid (BCS Amersham) the radioactivity of each fraction was counted in a Packard tricarb 2100TR liquid scintillation analyzer. The concentration of unlabeled steroid required to displace 50% of the bound [^{3}H]-DHT was calculated for DHT and for each complex by plotting the logit values of Y (logit $Y = \ln(Y/100 - Y)$ versus the mass of the competing complex. The RBA was calculated as follows: RBA of a compound = concentration of DHT required to displace 50% of [^{3}H]-DHT × 100/concentration of the compound required to displace 50% of [^{3}H]-DHT. The RBA value of DHT is by definition equal to 100%.

d. Measurement of Octanol/Water Partition Coefficient (log $P_{o/w}$) of the Compounds. The log $P_{o/w}$ values of the compounds were determined by reverse-phase HPLC on a C-8 column (Kromasil C8 from AIT) according to the method previously described by Minick[48] and Pomper.[49] Measurement of the chromatographic capacity factors (k') for each molecule was done at various concentrations in the range 95–80% methanol (containing 0.25% octanol) and an aqueous phase consisting of 0.15% *n*-decylamine in 0.02 M MOPS (3-morpholinopropanesulfonic acid) buffer pH 7.4 (prepared in 1-octanol-saturated water). These capacity factors (k') are extrapolated to 100% of the aqueous component given the value of k_w'. The log $P_{o/w}$ is then obtained by the formula $\log P_{o/w} = 0.13418 + 0.98452 \log k'$.

e. Culture Conditions. Cells were maintained in monolayer culture in DMEM with phenol red/Glutamax I, supplemented with 9% of decomplemented fetal calf serum and 0.9% kanamycine, at 37 °C in a 5% CO_2 air humidified incubator. For proliferation assays, cells were seeded at a density of 15000 to 25000 cells per millilter in 24-well sterile plates in 1 mL of DMEM without phenol red, supplemented with 9% of fetal calf serum desteroided on dextran charcoal, 0.9% Glutamax I and 0.9% kanamycine, and were incubated 24 h. The following day (D0), 1 mL of the same medium containing the compounds to be tested diluted in DMSO was added to the plates (four wells for each product). After 3 days (D3), the incubation medium was removed, and 2 mL of fresh medium containing the compounds was added. At different days (D3, D4, D5, and D6), the protein content of each well was quantified by methylene blue staining as follows. Cell monolayers were fixed and stained for 1 h in methanol with methylene blue (2.5 mg/mL) and then washed thoroughly with water. Two milliliters of HCl (0.1 M) was then added, and the plate was incubated for 1 h at 37 °C. Then the absorbance of each well was measured at 655 nm with a Biorad spectrophotometer (microplate reader). The results are expressed as the percentage of proteins versus the control. Experiments were performed at least in duplicate.

Acknowledgment. We thank the Ministère de la Recherche and the Centre National de la Recherche Scientifique for financial support, A. Cordaville for technical assistance, the Irish Council for Science, Engineering and Technology (IRCSET) for a France−Ireland Exchange, COST D39, and John Grealis for the 600 MHz NMR spectra.

Supporting Information Available: Numbering schemes for NMR assignment of compounds **3**, **5**, and **6**, crystallographic data for compounds **1** and **2**, and elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM701264D