Journal of Organometallic Chemistry 696 (2011) 1049–1056

Contents lists available at ScienceDirect

Journal of Organometallic Chemistry

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

Synthesis and biological activity of ferrocenyl derivatives of the non-steroidal antiandrogens flutamide and bicalutamide

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ARTICLE INFO

Article history:
Received 29 July 2010
Received in revised form
13 October 2010
Accepted 21 October 2010

Keywords:
Anticancer agents
Antiandrogen
Ferrocene
Prostate cancer
Flutamide
Bicalutamide

ABSTRACT

A series of ferrocenyl derivatives of the two non steroidal antiandrogens flutamide and bicalutamide have been prepared. Ferrocenyl bicalutamide complexes were initially synthesized in their racemic forms, and subsequently prepared as pure (*R*) and (*S*) enantiomers, and their structure was determined by X-ray crystallography. Most of the complexes retain a modest affinity for the androgen receptor and show an antiproliferative effect on both hormone-dependent (LNCaP) and -independent (PC-3) prostate cancer cells. Ferrocenyl derivatives of bicalutamide are the most cytotoxic (IC_{50} values on PC-3 around 15 μM); however, they are less potent than the ferrocenyl derivatives of ethynyltestosterone or nilutamide (IC_{50} around 5 μM).

1. Introduction

Prostate cancer is a major societal problem which affects one man in eight and is the cause of 200,000 deaths per year worldwide [1]. The growth and maintenance of the prostatic cells are stimulated by androgens, testosterone and 5α-dihydrotestosterone (DHT), the male sex hormones. The majority of these cancers are hormone dependent, and their development involves an interaction between the androgen receptor (AR) and the androgen [2]. The most important treatment of these hormone dependent cancers involves androgen withdrawal using surgical or chemical castration with steroidal or non-steroidal antiandrogens. Cyproterone acetate was one of the first steroidal antiandrogen clinically used but its side-effects, especially the interaction with the progestin and glucocorticoid receptor, made this drug less popular than the non-steroidal antiandrogens such as nilutamide [3,4], flutamide [5–7] and bicalutamide [8] (Chart 1). However, within a period of 1–3 years most patients relapse with advanced metastatic disease. The cancer usually becomes hormone refractory, possibly because of mutations of the androgen receptor [9], 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.

Moreover the antiandrogens described above are also antagonists of the AR in other peripheral tissues such as in muscle and bone in addition to prostate. In recent years, selective androgen receptor modulators (SARMs) have emerged whose aim is to target the AR in a tissue selective way, thus leading to selective biological effects [10,11]. For example, a SARM can be an antagonist or weak agonist in the prostate and fully agonistic in the pituitary and muscle. This concept of a SARM is thought to have therapeutic promise in the use of androgens [12]. The first generation of SARMs was discovered by Dalton and Miller [13,14] by replacing the sulfone of bicalutamide by an ether function, leading to SARM ***S*-1** (Chart 1). Since then, many SARMs have been reported in the literature such as arylpropionamide analogues [15] (derivatives of bicalutamide) and bicyclic hydantoins [16], and some of them are in now in the clinical phase [17].

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doi:10.1016/j.jorganchem.2010.10.051

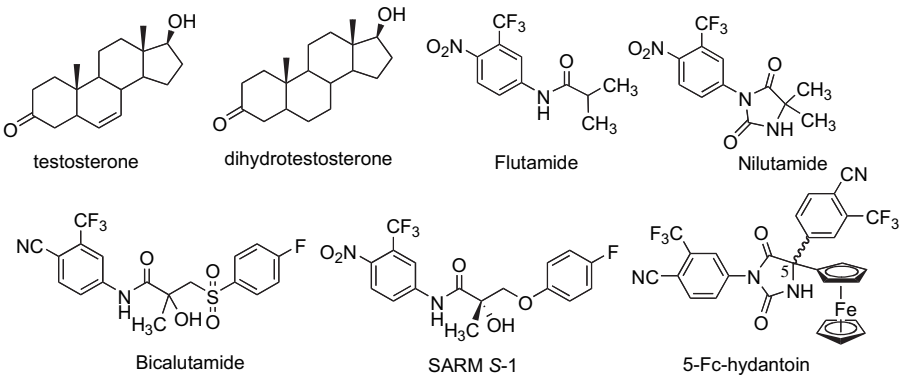


Chart 1.

We have previously shown that the incorporation of a ferrocenyl unit into non steroidal antiestrogens such as hydroxytamoxifen can lead to complexes showing a strong antiproliferative effect on both hormone dependent and -independent breast cancer cells [18–20]. Following the same strategy, we recently prepared ferrocenyl derivatives of nilutamide [21], (5-Fc-hydantoin) as well as of testosterone and dihydrotestosterone [22]. These new ferrocenyl derivatives showed strong cytotoxicity on hormone-independent PC-3 cell lines (IC_{50} around 5 μM). These very promising results prompted us to extend the study to ferrocenyl derivatives of flutamide and bicalutamide. Recently the synthesis of rhenium and technetium derivatives of flutamide was reported by Benny et al. as a novel class of single photon emission computer tomography (SPECT) imaging agents [23,24]. Our group also reported the synthesis of cyclopentadienyl-M-tricarbonyl derivatives (M = Re or Tc) of flutamide using the novel ferrocenyl derivative of flutamide **1** as the starting material (Chart 2) [25].

We here describe the syntheses of ferrocenyl derivatives of flutamide **2** and **3**, as well as ferrocenyl derivatives of SARM ***S*-1**: the racemate **4** and **5**, as well as the two pure enantiomers **(*R*)-5** and **(*S*)-5** (Chart 2). We also report some of their biological properties including the determination of their affinity for the androgen receptor, and an evaluation of their cytotoxic activities on hormone dependent (LNCaP) and hormone independent (PC-3) prostate cancer cells.

synthesis of **1** bearing a nitro substituent on the aromatic ring has recently been described [25] and the cyano analogue **2** was prepared following the same procedure, i.e. by treating the acid chloride formed from ferrocene carboxylic acid and oxalyl chloride with 4-cyano-3-trifluoromethyl-aniline in the presence of triethylamine to give *N*-(4-cyano-3-trifluoro-phenyl)-ferrocenylamide **2** in 48% yield (Scheme 1). Treatment of aminoferrocene with 2-methylpropanoyl chloride furnished *N*-(2-methylpropanoyl)-aminoferrocene, **3** in 43% yield.

The design of the ferrocenyl derivatives of SARM ***S*-1** involved the exchange of the fluorophenyl group by a ferrocenyl methyl unit while varying the *para*-substituent on the remaining aromatic with NO_2 and CN, thus leading to **4** and **5**, respectively. They were first obtained in racemic form before the independent syntheses of the two enantiomers **(*S*)-5** and **(*R*)-5** were carried out for comparison of their activity on prostate cancer cells.

The new compounds **4** and **5** were prepared via the reaction of sodium ferrocenylmethoxide with epoxides **8** and **9**, respectively, which were themselves obtained by following the literature procedure reported by Chen and coworkers [26] (Scheme 2). The reaction of 4-nitro-3-trifluoromethyl-fluorobenzene with sodium methacrylamide, generated *in situ* from the addition of NaH to methacrylamide, secured **6** in 88% yield. The epoxidation of **6**, performed in the presence of hydrogen peroxide and trifluoroacetic anhydride, furnished **8** in 61% yield. Analogously, **7** was obtained

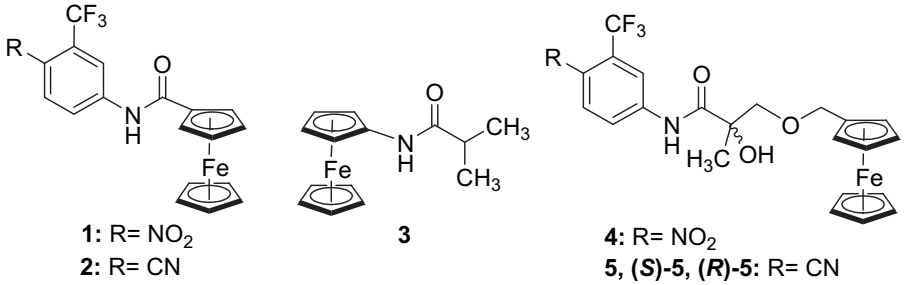
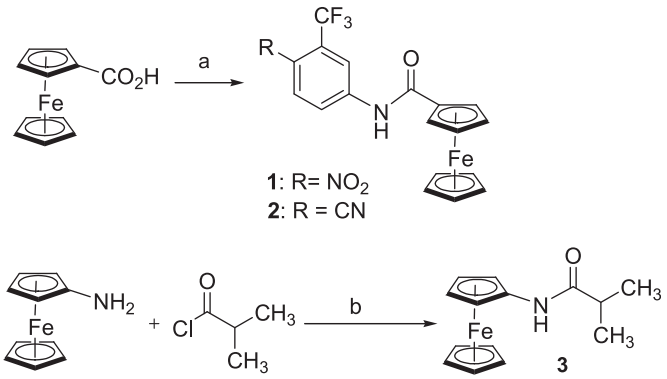


Chart 2.

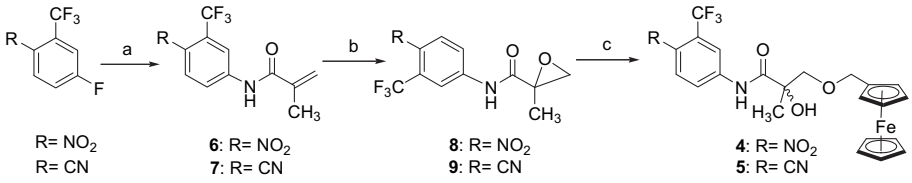
2. Results and discussion

2.1. Synthesis of the ferrocenyl derivatives of flutamide and bicalutamide *1*–*5*

A ferrocenyl moiety could be incorporated in the flutamide skeleton either by replacing its isopropyl group leading to the nitro and cyano compounds, **1** and **2**, or the aromatic ring to obtain **3**. The from 4-cyano-3-trifluoromethyl-fluorobenzene. The reaction of **7** with trifluoroacetic anhydride and hydrogen peroxide gave **9** in an improved yield of 87%. Finally, addition of **8** to sodium ferrocenylmethoxide, obtained from the reaction of NaH with ferrocenylmethanol in THF at 0 °C, led to the formation of **4** in 31% yield. Similarly, **5** was obtained in 28% yield by reaction of sodium ferrocenylmethoxide with **9**. The ring-opening of the epoxide was confirmed by the appearance in the ^{1}H NMR spectrum of



Scheme 1. Synthesis of ferrocenyl derivatives of flutamide. (a) (i) $(COCl)_2$, CH_2Cl_2, room temperature, 3 h, (ii) 4-nitro-3-fluoromethyl-aniline or 4-cyano-3-fluoromethyl-aniline, NEt_3, CH_2Cl_2, room temperature, 2 h, 40–48%; (b) NEt_3, CH_2Cl_2, room temperature, 2 h, 43%.

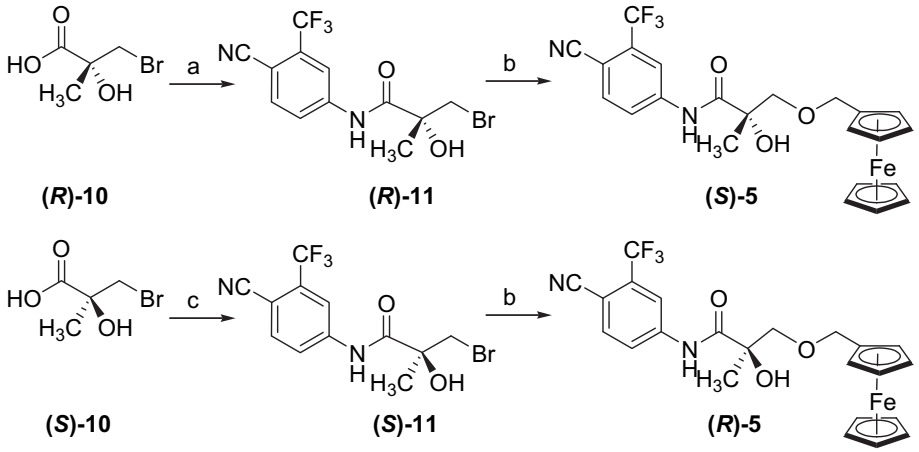


Scheme 2. Synthesis of the racemic derivatives of ferrocenyl SARM: (a) methacrylamide, NaH, DMF, room temperature, 3 h, 49–88%; (b) $(CF_3CO)_2O$, H_2O_2, CH_2Cl_2, reflux, 3 h, 61–87%; (c) (i) NaH, ferrocenylmethanol, THF, 0 °C, 5 min, (ii) **8** or **9**, reflux, 3 h, 31–28%.

diastereotopic protons adjacent to the ferrocenyl unit. Thus, in **5**, the CH_2Fc protons appeared as 11.3 Hz doublets at 4.41 and 4.31 ppm.

Following the successful preparation of **5** as a racemate, the synthesis of its two enantiomers was explored. **(*S*)-5** was prepared from (*R*)-proline, which was transformed into (*R*)-2-hydroxy-3-bromo-propanoic acid **(*R*)-10** in a three-step process following the method used to prepare (*R*)-bicalutamide, as described by Kirkovsky et al. [27] (Scheme 3). Treatment of **(*R*)-10** with thionyl chloride in THF gave the corresponding acyl chloride; subsequent reaction with 4-cyano-3-trifluoromethyl-aniline and triethylamine led to **(*R*)-11**, as reported by Miller and coworkers for the formation of SARM ***S*-1** [28]. Finally, the reaction of **(*R*)-11** with sodium ferrocenylmethoxide completed the synthesis of the ferrocenyl derivative **(*S*)-5** in 31% yield. Analogously, the route to the pure enantiomer **(*R*)-5**, followed the same synthetic method as was used for the preparation of (*R*)-2-hydroxy-3-bromo-propanoic acid **(*R*)-10**, but starting this time from (*S*)-Proline (Scheme 3). The brominated derivative **(*S*)-11** was then formed from **(*S*)-10** by a slightly different procedure in that THF was replaced by DMA, and no triethylamine was added, as reported for the synthesis of (*R*)-bicalutamide [29]. The final step, the reaction of **(*S*)-11** with sodium ferrocenylmethoxide to form **(*R*)-5** proceeded in 37% yield. The formation of the two enantiomers **(*S*)-5** and **(*R*)-5** was confirmed by the measurement of their optical rotations which gave specific rotations of −10° and +11°, respectively.

It is noteworthy that during the formation of the brominated compounds **(*R*)-10** and **(*S*)-10**, the corresponding chlorinated molecules are also formed to the extent of 7–14%, as reported by Kirkovsky [27]. This mixture of halogenated compounds subsists for **(*R*)-11** and **(*S*)-11** but does not interfere in the formation of **(*S*)-5** and **(*R*)-5**.



Scheme 3. Synthesis of the two enantiomers of ferrocenyl SARM: (a) (i) $SOCl_2$, THF, −5 °C, 2 h, and (ii) 4-amino-2-trifluoromethylbenzonitrile, NEt_3, THF, reflux, 12 h, 55%; (b) (i) K_2CO_3, acetone, reflux, 4 h, (ii) ferrocenylmethanol, NaH, THF, room temperature, 12 h, 31–37%; (c) (i) $SOCl_2$, DMA, −5 °C, 30 min, (ii) 4-amino-2-trifluoromethylbenzonitrile, DMA, room temperature, 3 h, 32%.

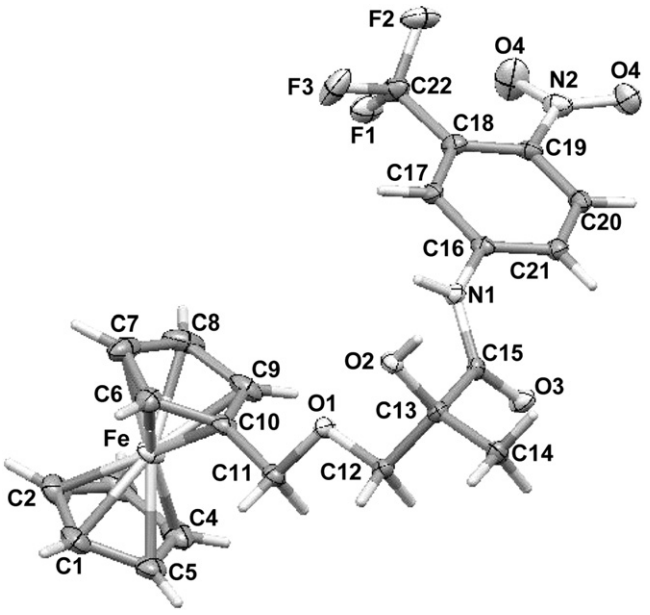


Fig. 1. X-ray crystal structure of **4**; thermal ellipsoids are drawn at the 50% probability level. Selected bond lengths (Å) and angles (deg): Fe-Cent(1) 1.645(2); Fe-Cent(2) 1.635 (2); C9–C10 1.423(3); C1–C2 1.419(3); C10–C11 1.487(3); C11–O1 1.435(2); O1–C12 1.420(2); C12–C13 1.522(3); C13–C15 1.542(3); C15–N1 1.357(3); N1–C16 1.408(2); C10–C11–O1 106.92(15); C11–O1–C12 112.79(14); O1–C12–C13 107.64(15); C12–C13–C15 107.48(15); C13–C15–N1 115.71(16); C15–N1–C16 124.16(16).

2.2. X-ray crystal structure of **4**

The structure of racemic **4** was confirmed by X-ray crystallography, and Fig. 1 shows only the (*S*) enantiomer. Crystallographic data are collected in Table 1. The structural parameters for the ferrocenyl group are within the normal ranges, and the iron atom is sandwiched almost perfectly centrally between the two cyclopentadienyl rings. The side-chain bearing the nitro-trifluoromethyl-phenyl adopts a distal position with respect to the C_5H_5 ring.

Table 1
Crystal data and structure refinement for compound **4**.

Empirical formula	$C_{22}H_{21}N_2O_5F_3Fe$
Formula weight	506.26
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P−1 (#2)
Unit cell dimensions	$a = 9.5084(9)$ Å, $\alpha = 67.137(2)^\circ$.
	$b = 11.3147(11)$ Å, $\beta = 76.914(2)^\circ$.
	$c = 11.3550(11)$ Å, $\gamma = 74.081(2)^\circ$.
Volume	1072.53(18) Å^3
Z	2
Density (calculated)	1.568 Mg/m^3
Absorption coefficient	0.767 mm^{-1}
F(000)	520
Crystal size	0.40 × 0.30 × 0.28 mm^3
Theta range for data collection	1.96–26.00°
Index ranges	$-11 \le h \le 11$, $-13 \le k \le 13$, $-13 \le l \le 13$
Reflections collected	8771
Independent reflections	4116 [*R*(int) = 0.0168]
Completeness to theta = 26.00°	97.7%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.8139 and 0.6331
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	4116/0/307
Goodness-of-fit on F^2	1.068
Final *R* indices [$I > 2$sigma(I)]	$R1 = 0.0352$, $wR2 = 0.0902$
R indices (all data)	$R1 = 0.0384$, $wR2 = 0.0931$
Largest diff. peak and hole	0.827 and −0.321 eÅ^{-3}

2.3. Biochemical studies

The biological properties of the ferrocenyl derivatives **1**–**5** were tested and compared with those obtained with the male steroids dihydrotestosterone (DHT), and the non-steroidal antiandrogens bicalutamide and hydroxy-flutamide, the active metabolite of flutamide. The relative binding affinity (RBA) values of the compounds for the androgen receptor (AR) were assayed in a standard competitive radio ligand assay, using full-length human recombinant androgen receptor (AR) and [^{3}H]-DHT as a tracer. The RBA values obtained are reported in Table 2, and are shown to be very low (<0.3%) even for bicalutamide and hydroxy flutamide, the antiandrogens used for the treatment of prostate cancer. This is the case for most of the non-steroidal antiandrogens and could be explained by the fact that their chemical structures have only a poor analogy with those of the natural male steroids, testosterone and DHT. In both series, RBA values are higher for the nitro than the cyano complexes and those of the ferrocenyl complexes are lower than that of their organic counterparts. Decreases are limited in the bicalutamide series but significant in the flutamide one where complex **2** has completely lost its recognition for the AR. This is also the case for complex **3** where a nonsubstituted ferrocenyl replaces a flat disubstituted aromatic ring. This confirms the observation that CF_3 and NO_2 groups (or CN) play an important role in the recognition of the ligand by its specific receptor. Indeed, it has been shown that the cyano group of bicalutamide forms H bonds with Gln711 and Arg752 of androgen receptor ligand binding domain while the CF3 occupies a hydrophobic pocket [2,14].

The effect of 10 μM of the newly synthesized molecules was then studied on the growth of both hormone-dependent (LNCaP) and hormone-independent (PC-3) prostate cancer cells, and the results are also shown in Table 2. On LNCaP cells, DHT and hydroxy-flutamide have a proliferative effect, bicalutamide has almost no effect, while all the ferrocenyl complexes show an antiproliferative effect. The proliferative effect found for hydroxy flutamide described as an antiandrogen is quite surprising, and it was also found with nilutamide on this cell line [30–32]. This could be attributable to the fact that the AR present in LNCaP cells contains a point mutation in the ligand-binding domain [33]. Unfortunately, however, it is the only available human cell line that shows both hormone dependency and continuous growth *in vitro*.

Owing to the low RBA values found for the complexes, the observed antiproliferative effect might be associated with a cytotoxic rather to an anti-hormonal effect. On the hormone-independent prostate cancer cells PC-3, five ferrocenyl complexes show an antiproliferative effect higher than 15%, while the other compounds, including the organic molecules hydroxy-flutamide, bicalutamide and SARM *S*-1, and two of three ferrocenyl derivatives of flutamide (**2** and **3**) have almost no effect. IC_{50} values were determined for the five most cytotoxic complexes and are reported in Table 2. They range from 30.5 μM for the nitro ferrocenyl flutamide derivative **1** to an average of 13 μM for the three cyano ferrocenyl bicalutamide derivatives **5** (racemic, (*S*) and (*R*) forms). The ferrocenyl complex **4** is significantly more cytotoxic than its corresponding organic molecule SARM *S*-1, but it seems that the stereochemistry of the asymmetric carbon plays no role in the cytotoxic effect of the complexes as **5**, **(*S*)-5** and **(*R*)-5** exhibit similar IC_{50} values. This result is different from what is observed *in vivo* for the antiandrogenic effect of the two bicalutamide enantiomers. In that case, the enantiomer with the (*R*) absolute configuration has been described as being 60-fold more potent than the (*S*) enantiomer [29], even if prescribed as the racemic mixture [34].

Table 2
Relative Binding Affinity (RBA) for the androgen receptor (AR), effect on the growth of LNCaP (hormone-dependent prostate cancer cells) and of PC-3 (hormone-independent prostate cancer cells) and log $P_{o/w}$ of the compounds.

Compound	RBA (%) on AR	Cell viability on LNCaP (%)[a]	Cell viability on PC-3[a] (%)	IC_{50} on PC-3 (μM)[b]	log $P_{o/w}$
DHT	100	148	99	–	3.2
OH-flutamide	0.20 ± 0.01	181 ± 47	87 ± 3	–	3.75
1	0.007 ± 0.004	78 ± 7	71 ± 2	30 ± 1	4.42
2	0.00	83 ± 12	86.2 ± 0.6	–	4.10
3	0.00	89 ± 2	92.4 ± 0.6	–	2.64
Bicalutamide	0.29 ± 0.02	93 ± 4	85 ± 6	–	3.40
SARM *S*-1	nd	nd	94 ± 3	–	–
4	0.084 ± 0.02	73 ± 11	65 ± 3	17 ± 2	5.01
5	0.040 ± 0.006	73 ± 18	76 ± 2	13.5 ± 0.3	4.63
(*S*)-5	nd	55 ± 1.5	83 ± 1	12.8 ± 0.5	4.82
(*R*)-5	nd	nd	81 ± 4	14 ± 2	4.69

[a] Incubation in the presence of 10 μM of the compounds [except for DHT (10 nM), OH-flutamide (1 μM) on LNCaP], after 5 days of culture. Non-treated cells are used as the control (100%). An effect higher than 100% indicates a proliferative effect. Mean of two separate experiments (6 measurements for each one) ± range.
[b] IC_{50} values are determined when cell viability in the presence of 10 μM of the complex is lower than 85% (with the control set at 100%).

Finally, the lipophilicity values found for the new molecules are higher than the log $P_{o/w}$ values of bicalutamide or hydroxy flutamide (except for **3**) (Table 2). This was to be expected as they all possess ferrocenyl substituents which are known to be lipophilic.

3. Conclusions

The results found here confirm that the ferrocenyl derivatives of flutamide and of bicalutamide are only weakly recognized by the androgen receptor, but this is not a complete surprise considering that there is not a close analogy between their structures and those of the natural androgens, testosterone and dihydrotestosterone. The ferrocenyl complexes of bicalutamide are noticeably more cytotoxic towards the hormone-independent cell line PC-3 than are those of ferrocenyl flutamide. With an IC_{50} value of 17 μM, the ferrocenyl complex **4** is considerably more cytotoxic than the corresponding organic compound SARM *S*-1. In contrast, however, the absolute stereochemistry (*R* or *S*) of these complexes does not appear to play any significant role. Finally, the IC_{50} values found for the ferrocenyl bicalutamide derivatives **4** and **5**, the most active compounds of the series, are significantly higher than the values (around 5 μM) found previously for the ferrocenyl complexes of ethynyl testosterone or nilutamide.

4. Experimental section

4.1. General comments

All reactions were carried 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. ^1H and ^{13}C NMR spectra were recorded on a 300 MHz Bruker spectrometer. 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, λ = 254 nm.

4.2. Synthesis

The synthesis of SARM ***S*-1** was carried out following the literature procedure [14] as well as **(*R*)-10** and **(*S*)-10** [27,29], **1** [25], **6** and **7** [8,26] and hydroxy-flutamide [35].

4.2.1. *N*-(4-cyano-3-trifluoromethyl-phenyl)-ferrocenyl-carboxamide (**2**)

Ferrocenecarboxylic acid (0.460 g, 2.00 mmol) was partially dissolved in 20 mL of dichloromethane. Oxalyl chloride (0.35 mL, 4.00 mmol) was added dropwise followed by one drop of DMF. After stirring for 3 h at room temperature, dichloromethane and excess of oxalyl chloride were removed under reduced pressure. Dichloromethane (20 mL) was added to dissolve the acid chloride formed. In another Schlenk tube, 4-cyano-3-trifluoromethyl-aniline (0.372, 2.00 mmol) was dissolved in 20 mL of dichloromethane and triethylamine (0.28 mL, 2.00 mL) was added. The solution was cooled to 0 °C, then the solution of acid chloride was added dropwise. The mixture was stirred at room temperature for 2 h before being poured in water. The reaction mixture was extracted with dichloromethane. The organic layer was washed with water, dried over $MgSO_4$, filtered, and solvents evaporated. The crude product obtained was purified by chromatography on silica gel column (eluent:ethyl acetate/petroleum ether 1:3). Compound **2** was isolated as an orange solid which was crystallized in ethyl acetate:-pentane (0.383 g, 48% yield): mp: 205 °C; ^1H NMR (300 MHz, [d_6] acetone): δ = 9.45 (s, 1H, NH), 8.43 (d, J = 2.0 Hz, 1H, Ar H_2,), 8.27 (dd, J = 8.4 , J = 2.0 Hz, 1H, Ar H_6,), 7.99 (d, J = 8.4 Hz, 1H, Ar H_5), 5.01 (t, J = 2.0 Hz, 2H, C_5H_4), 4.51 (t, J = 2.0 Hz, 2H, C_5H_4), 4.25 (s, 5H, C_5H_5); ^{13}C NMR (75 MHz, [d_6]acetone): δ = 69.7 (C_5H_4), 70.7 (C_5H_5), 72.2 (C_5H_4), 76.1 (C_5H_4, C_{ip}), 104.0 (Ar C_4), 116.5 (CN), 118.0 (Ar C_2), 123.0 (Ar C_6), 124.0 (q, CF_3, J = 262.5 Hz), 134.0 (Ar C_3), 137.0 (Ar C_5), 144.9 (Ar C_1), 171.2 (CO); IR (CH_2Cl_2): $\nu \simeq$ 2230 (CN), 1687 (CO) cm^{-1}; MS (EI) m/z: 398 [$M^{+\cdot}$]; Anal. Calcd for $C_{19}H_{13}F_3FeN_2O$: C 57.31, H 3.29, N 7.04, found: C 57.16, H 3.43, N 7.22.

4.2.2. *N*-(ferrocenyl)-isobutyramide (**3**)

Aminoferrocene (0.310 g, 1.54 mmol) was dissolved in 20 mL of dichloromethane. Triethylamine (0.22 mL, 1.54 mmol) was added into the solution. The mixture was cooled to 0 °C, then isobutyryl chloride (0.16 mL, 1.54 mmol) was added dropwise. After stirring for 2 h at room temperature, the mixture was poured in water and extracted with dichloromethane. The organic layer was then washed with water, dried over $MgSO_4$, filtered, and evaporated. The crude product obtained was purified by chromatography on silica gel column (eluent:dichloromethane/petroleum ether 3:1). Compound **3** was isolated as an orange solid which was crystallized in dichloromethane:pentane (0.179 g, 43% yield): mp: 172–174 °C; ^1H NMR (300 MHz, $CDCl_3$): δ = 6.43 (s, 1H, NH), 4.65 (s, 2H, C_5H_4), 4.16 (s, 5H, C_5H_5), 4.03 (s, 2H, C_5H_4), 2.39 (septuplet, J = 6.9 Hz, 1H, CH), 1.23 (s, 3H, CH_3), 1.20 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 19.8 (CH_3), 36.4 (CH), 61.4 (C_5H_4), 64.8 (C_5H_4), 69.6 (C_5H_5), 95.9 (C_5H_4, C_{ip}); IR (CH_2Cl_2): $\nu \simeq$ 1733 cm^{-1} (CO); MS (EI) m/z: 271 [$M^{+\cdot}$]; Anal. Calcd for $C_{14}H_{17}FeNO$: C 62.02, H 6.32, N 5.17, found: C 61.96, H 6.31, N 5.29.

4.2.3. N-(4-nitro-3-trifluoromethyl-phenyl)-3-ferrocenylmethoxy-2-hydroxy-2-methyl- propanamide (**4**)

A solution of ferrocenylmethanol (0.610 g, 2.82 mmol) in THF (10 mL) was added to a suspension of NaH in THF (0.226 g, 5.64 mmol) at 0 °C. The mixture was stirred for 5 min. A solution of **8** (0.500 g, 2.17 mmol) in THF (15 mL) was added slowly. The mixture was stirred at reflux for 3 h, and then cooled to room temperature. THF was then distilled off followed by addition of ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, filtered and evaporated. The crude product was purified by chromatography on silica gel column (eluent:ethyl acetate/petroleum ether 1:3, then 1:2). Compound **4** was obtained as a red oil (0.273 g, 31 % yield). The oil was crystallized from dichloromethane/hexane to give an orange solid: mp: 146–148 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 9.10$ (s, 1H, NH), 7.95–7.99 (m, 3H, Ar H_2, H_5, H_6), 4.24–4.34 (m, 6H, $C_5H_4 + CH_2Fc$), 4.21 (s, 5H, C_5H_5), 3.84 (d, $J = 9.0$ Hz, 1H, CH_2O), 3.46 (s, 1H, OH), 3.41 (d, $J = 9.0$ Hz, 1H, CH_2O), 1.39 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 23.2$ (CH_3), 69.4 (C_5H_5), 69.8 (C_5H_4), 70.2 (CH_2), 70.3 (C_5H_4), 73.9 (CH_2), 75.4 (*C*(OH)), 82.9 (C_5H_4, C_{ip}), 118.3 (Ar C_2), 121.8 (q, CF_3, $J = 266.5$ Hz), 122.1 (Ar C_6), 125.2 (q, Ar C_3), 127.2 (Ar C_5), 141.8 (Ar C_1) 143.2 (Ar C_4), 174.0 (CO); IR (KBr): $\nu \simeq 3523$ (OH), 3306 (NH), 1696 (CO) cm^{-1}; MS (EI) *m*/*z*: 506 [M$^{+\cdot}$]. Anal. Calcd for $C_{22}H_{21}F_3FeN_2O_5$: C 52.19, H 4.18, N 5.53, found: C 52.23, H 4.24, N 5.49.

4.2.4. N-(4-cyano-3-trifluoromethyl-phenyl)-3-ferrocenylmethoxy-2-hydroxy-2-methyl-propanamide (**5**)

A solution of ferrocenylmethanol (0.520 g, 2.4 mmol) in THF (10 mL) was added to a suspension of NaH (0.192 g; 4.8 mmol) in THF at 0 °C. The mixture was stirred for 5 min. A solution of **9** (0.500 g; 1.85 mmol) in THF (12 mL) was added slowly. The mixture was stirred at reflux for 2.5 h, then cooled to room temperature. THF was then distilled off before ethyl acetate was added. The organic layer was washed with water, dried over $MgSO_4$, filtered and evaporated. The crude solid obtained was purified by chromatography on silica gel column (eluent:ethyl acetate/petroleum ether 1:2). Compound **5** was obtained as a yellow solid (0.250 g, 28.0% yield). The solid was recrystallized from dichloromethane/hexane: mp: 102–104 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 9.09$ (s, 1H, NH), 8.01 (d, $J = 1.6$ Hz, 1H, Ar H_2), 7.88 (dd, $J = 8.5$, $J = 1.6$ Hz, 1H, Ar H_6), 7.77 (d, $J = 8.5$ Hz, 1H, Ar H_5), 4.41 (d, $J = 11.3$ Hz, 1H, CH_2Fc), 4.31 (d, $J = 11.3$ Hz, 1H, CH_2Fc), 4.21 (s, 2H, C_5H_4), 4.19 (s, 2H, C_5H_4), 4.14 (s, 5H, C_5H_5), 3.87 (d, $J = 9.0$ Hz, 1H, CH_2O), 3.49 (s, 1H, OH), 3.44 (d, $J = 9.0$ Hz, 1H, CH_2O), 1.40 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 23.3$ (CH_3), 68.8 (C_5H_5), 69.2 (C_5H_4), 69.8 (C_5H_4), 70.3 (CH_2), 74.0 (CH_2), 75.6 (*C*(OH)), 82.3 (C_5H_4, C_{ip}), 104.6 (Ar C_4), 115.8 (CN), 117.4 (Ar C_2), 122.0 (Ar C_6), 134.0 (q, Ar C_3), 136.0 (Ar C_5), 141.8 (Ar C_1), 174.1 (CO); IR (KBr): $\nu \simeq 3410$ (OH), 3311 (NH), 2232 (CN), 1691 (CO) cm^{-1}; MS (EI) *m*/*z*: 486 [M$^{+\cdot}$]; Anal. Calcd for $C_{23}H_{21}F_3FeN_2O_3$: C 56.81, H 4.35, N 5.76, found: C 56.41, H 4.72, N 5.31.

4.2.5. (2S)-N-(4-cyano-3-trifluoromethylphenyl)-3-ferrocenylmethoxy-2-hydroxy-2-methylpropionamide (**(S)-5**)

Under argon, (2*R*)-3-bromo-*N*-(4-cyano-3-trifluoromethylphenyl)-2-hydroxy-2-ethylpropionamide **(*R*)-11** (0.296 g, 0.843 mmol) and potassium carbonate (0.261 g, 1.89 mmol) were dissolved in acetone (3 mL) and the reaction mixture was refluxed for 4 h. After dilution in 40 mL of water, the mixture was extracted with ethyl acetate (3 × 30 mL), the organic phase was dried over sodium sulfate and solvents evaporated under vacuum. A yellow-brownish oil corresponding to the epoxide was obtained and used without further purification. In the meantime, a solution of ferrocenylmethanol (0.28 g, 1.30 mmol) in distilled THF (4 mL) was added to a suspension of sodium hydride (0.063 g, 2.63 mmol) in THF (2 mL) under argon. The reaction mixture was stirred at 60 °C for 1 h before the epoxide dissolved in distilled THF (2 mL) was added. The mixture was stirred at reflux for 2 h before it was left stirring at room temperature for 12 h. The mixture was then quenched by addition of water (30 mL) and was then extracted with ethyl acetate (4 × 30 mL). After drying over magnesium sulfate, the organic phase was concentrated under vacuum to give a brown oil. It was then purified by column chromatography on silica gel (eluent:petroleum ether/ethyl acetate 3:2) to give the pure product as a light brown solid (0.128 g, 31% yield): mp: degradation above 50 °C; $[\alpha]_D^{20} = -10$ ($c = 1.9$ in acetone); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 9.11$ (sl, 1H, NH); 7.98 (d, $J = 1.3$ Hz, 1H, Ar H_2), 7.83 (d, $J = 8.7$, $J = 1.7$, Hz, 1H, Ar H_6), 7.69 (d, $J = 8.5$ Hz, 1H, Ar H_5), 4.40 (d, $J = 11.1$ Hz, 1H, CH_2Fc), 4.31 (1H, d, $J = 11.1$ Hz, CH_2Fc), 4.20–4.14 (m, 4H, C_5H_4), 4.11 (s, 5H, C_5H_5), 4.01 (d, $J = 9.0$ Hz, 1H, CH_2O), 3.60 (s, 1H, OH), 3.43 (d, $J = 9.0$ Hz, 1H, CH_2O), 1.38 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 23.0$ (CH_3), 68.6 (C_5H_5), 69.0 (C_5H_4), 69.6 (C_5H_4), 70.1 (CH_2), 74.1 (CH_2), 75.4 (C(OH)), 82.0 (C_5H_4, C_{ip}), 104.1 (Ar C_4), 115.5 (CN), 117.2 (Ar C_2), 120.3 (CF_3), 121.7 (Ar C_6), 133.8 (q, Ar C_3,), 135.7 (Ar C_5), 141.6 (Ar C_1), 173.9 (CO); FT-IR: $\nu \simeq 3497$ (OH), 3324 (NH), 2231 (CN), 1700 (CO) cm^{-1}; MS (ESI) *m*/*z*: 486 [M$^{+\cdot}$]; Anal. Calcd for $C_{23}H_{213}F_3FeN_2O_3$: C 56.81, H 4.35, N 5.76, found: C 56.80, H 4.54, N 5.66.

4.2.6. (2R)-N-(4-cyano-3-trifluoromethylphenyl)-3-ferrocenylmethoxy-2-hydroxy-2-methylpropionamide ((**R)-5**)

Under argon, (2*S*)-3-bromo-*N*-(4-cyano-3-trifluoromethylphenyl)-2-hydroxy-2-methylpropionamide **(*S*)-11** (0.296 g, 0.84 mmol) and potassium carbonate (0.261 g, 1.89 mmol) were dissolved in acetone (3 mL) and the reaction mixture was refluxed for 4 h. After dilution in 40 mL of water, the mixture was extracted with ethyl acetate (3 × 30 mL), the organic phase was dried over sodium sulfate and solvents evaporated under vacuum. A yellow-brownish oil corresponding to the epoxide was obtained and used without further purification. In the meantime, a solution of ferrocenylmethanol (0.28 g, 1.30 mmol) in distilled THF (4 mL) was added to a suspension of sodium hydride (0.063 g, 2.63 mmol) in THF (2 mL) under argon. The reaction mixture was stirred at 60 °C for 1 h before the epoxide dissolved in distilled THF (2 mL) was added. The mixture was stirred at reflux for 2 h before it was left stirring at room temperature for 12 h. The mixture was then quenched by addition of water (30 mL) and was then extracted with ethyl acetate (4 × 30 mL). After drying over magnesium sulfate, the organic phase was concentrated under vacuum to give a brown oil. It was then purified by column chromatography on silica gel (eluent:petroleum ether/ethyl acetate 3:2) to give the pure product as a light brown solid (0.15 g, 37% yield): mp: degradation above 50 °C; $[\alpha]_D^{20} = +11°$ ($c = 1.9$ in acetone); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 9.06$ (broad singlet, 1H, NH), 7.99 (s, 1H, Ar H_2), 7.85 (d, $J = 8.1$ Hz, 1H, Ar H_6), 7.73 (d, $J = 8.0$ Hz, 1H, Ar H_5), 4.20–4.09 (m, 6H, C_5H_4, CH_2Fc), 4.06 (s, 5H, C_5H_5), 3.84 (d, $J = 9.0$ Hz, 1H, CH_2O), 3.45 (1H, s, OH), 3.41 (d, $J = 9.0$ Hz, 1H, CH_2O), 1.37 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 23.0$ (CH_3), 69.5 (C_5H_5), 69.9 (C_5H_4), 70.0 (C_5H_4), 70.4 (C_9), 73.9 (C_8), 75.3 (C(OH)), 82.9 (C_5H_4, C_{ip}), 104.3 (Ar C_4), 115.5 (CN), 117.2 (Ar C_2), 120.3 (Ar CF_3), 121.7 (Ar C_6), 133.8 (q, Ar C_3,), 135.8 (Ar C_5), 141.6 (Ar C_1), 173.8 (CO); FT-IR: $\nu \simeq 3473$ (OH), 3319 (NH), 2231 (CN), 1700 (CO) cm^{-1}; MS (ESI) *m*/*z*: 486 [M$^{+\cdot}$]; Anal. Calcd for $C_{23}H_{213}F_3FeN_2O_3$: C 56.81, H 4.35, N 5.76, found: C 56.88, H 4.72, N 5.37.

4.2.7. N-(4-nitro-3-trifluoromethyl-phenyl)-methyl-oxiranyl-carboxamide (**8**)

To a stirred solution of **6** (2.00 g, 7.30 mmol) in dichloromethane (20 mL) was added an aqueous solution of 30% hydrogen peroxide H_2O_2 (1.4 mL; 43.8 mmol). Trifluoroacetic anhydride (5.2 mL; 36.5 mmol) was added slowly and the reaction mixture was stirred to reflux for 3 h. The mixture was poured in dichloromethane. The organic layer was then washed with water, saturated aqueous sodium bisulfite $NaHSO_3$, saturated sodium

bicarbonate, brine, dried over $MgSO_4$, filtered, and evaporated. The crude product obtained was purified by chromatography on silica gel column (eluent:dichloromethane/petroleum ether 3:1). Compound **8** was obtained as a pale yellow solid (1.30 g, 61% yield): mp: 120–122 °C (literature [8]: 120–121 °C); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.48$ (s, 1H, NH), 8.01–7.96 (m, 3H, Ar H_2, H_5, H_6), 3.01 (s, 2H, CH_2O), 1.68 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 16.9$ (CH_3), 54.4 (CH_2), 56.9 ($C(CH_3)$), 118.5 (Ar C_2), 121.9 (q, $J = 273$ Hz, CF_3), 122.3 (Ar C_6), 125.6 (q, $J = 34.2$ Hz, Ar C_3), 127.3 (Ar C_5), 141.5 (Ar C_1), 143.5 (Ar C_4), 169.5 (CO); IR (KBr): $\nu \simeq 3339$ (NH), 1708 (CO) cm^{-1}.

4.2.8. N-(4-cyano-3-trifluoromethyl-phenyl)-methyl-oxiranyl-carboxamide [26] (**9**)

To a stirred solution of **7** (0.500 g, 1.97 mmol) in dichloromethane (10 mL) was added an aqueous solution of 30% hydrogen peroxide H_2O_2 (0.40 mL; 6.00 mmol). Trifluoroacetic anhydride (1.4 mL; 9.85 mmol) was added slowly and the reaction mixture was stirred at room temperature for 2 h 30 s. The mixture was poured in dichloromethane. The organic layer was then washed with water, saturated aqueous sodium bisulfite $NaHSO_3$, saturated sodium bicarbonate, brine, dried over $MgSO_4$, filtered, and concentrated under vacuum. Compound **9** was isolated as a white solid (0.465 g; 87% yield): mp: 147–148 °C (literature [26]: 149–150 °C); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.44$ (s, 1H, NH), 8.02 (d, $J = 2.1$ Hz, 1H, Ar H_2), 7.90 (dd, $J = 8.7$, $J = 2.1$ Hz, 1H, Ar H_6), 7.78 (d, $J = 8.7$ Hz, 1H, Ar H_5), 2.99 (s, 2H, CH_2O), 1.67 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 16.8$ (CH_3), 54.2 (CH_2), 56.8 ($C(CH_3)$), 104.8 (Ar C_4), 115.5 (CN), 117.4 (Ar C_2), 122.0 (Ar C_6), 122.1 (q, $J = 273.9$ Hz, CF_3), 134.0 (q, $J = 33.0$ Hz, Ar C_3), 136.0 (Ar C_5), 141.4 (Ar C_1), 169.4 (CO); IR (KBr): $\nu \simeq 3334$ (NH), 2230 (CN), 1706 (CO) cm^{-1}.

4.2.9. (2R)-N-(4-cyano-3-trifluoromethylphenyl)-3-bromo-2-hydroxy-2-methylpropionamide [28] (**(R)-11**)

Under argon, a solution of (2*R*)-3-bromo-hydroxy-2-methyl-propanoic acid (0.502 g, 2.74 mmol) in distilled THF (5 mL) was cooled to −5 °C before thionyl chloride (0.21 mL, 2.88 mmol) was added dropwise. The reaction mixture was left stirring at −5 °C for 2 h. A solution of 4-amino-2-trifluoromethylbenzonitrile (0.470 g, 2.28 mmol) in distilled THF (5 mL) was then added at −5 °C followed by distilled triethylamine (0.87 mL, 6.24 mmol). The reaction was warmed to room temperature and was then refluxed for 12 h. The mixture was then diluted in ethyl acetate (50 mL), washed with diluted HCl 5% (3 × 30 mL), saturated aq. $NaHCO_3$ (3 × 30 mL) and water (3 × 30 mL). The organic phase was dried over magnesium sulfate and the solvents evaporated. The dark oil obtained was purified by column chromatography on silica gel (eluent:petroleum ether/ethyl acetate, 3:2) to give a brown solid (0.441 g, 55% yield): mp = 135 °C (literature [27]: 132–134 °C). The NMR spectrum shows the presence of 93% brominated compound and 7% of chlorinated one: ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 9.02$ (sl, 1H, NH). 8.07 (d, $J = 1.8$ Hz, 1H, Ar H_2), 7.97 (dd, $J = 8.7$, $J = 2.1$ Hz, 1H, Ar H_6), 7.77 (d, $J = 8.4$ Hz, 1H, Ar H_5), 4.12 (d, $J = 10.8$ Hz, 1H, CH_2Cl), 4.02 (d, $J = 10.8$ Hz, 1H, CH_2Br), 3.67 (d, $J = 10.8$ Hz, 1H, CH_2Cl), 3.57 (d, $J = 10.8$ Hz, 1H, CH_2Br), 3.07 (s, 1H, O*H*(Cl)), 3.02 (s, 1H, O*H*(Br)), 1.62 (s, 3H, CH_3(Br)), 1.57 (s, 3H, CH_3(Cl)).

4.2.10. (2S)-N-(4-cyano-3-trifluoromethylphenyl)- 3-bromo-2-hydroxy-2-methylpropionamide [29] (**(S)-11**)

Under argon, a solution of (2*S*)-3-bromo-hydroxy-2-methyl-propanoic acid (0.2 g, 1.09 mmol) in anhydrous DMA (3 mL) was cooled to 5 °C before thionyl chloride was added dropwise. The reaction mixture was stirred for 30 min at this temperature and a solution of 4-amino-2-trifluoromethylbenzonitrile (0.183 g, 0.983 mmol) was added rapidly to the solution containing the acid chloride formed *in situ*. The reaction mixture was left stirring for 3 h at room temperature. DMA was then removed under vacuum and the dark residue obtained was diluted with saturated aqueous $NaHCO_3$ (5 mL) and the solution was extracted with ether (3 × 10 mL). The organic phase was dried over magnesium sulfate and concentrated under vacuum. The dark oil obtained was purified by column chromatography on silica gel (eluent:petroleum ether/ethyl acetate, 3:2) to give a brown solid (0.112 g, 32% yield): mp: 106 °C (literature [29]: 106–107 °C); The NMR spectrum shows the presence of 86% brominated compound and 14% of chlorinated one: ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 9.08$ (broad singlet, 1H, NH), 8.11 (s, 1H, Ar H_2), 7.96 (d, $J = 8.7$ Hz, 1H, Ar H_6), 7.80 (d, $J = 8.1$ Hz, 1H, Ar H_5), 4.10 (d, $J = 10.8$ Hz, 1H, CH_2Cl), 4.05 (d, $J = 10.8$ Hz, 1H, CH_2Br), 3.69 (d, $J = 10.8$ Hz, 1H, CH_2Cl), 3.59 (d, $J = 10.8$ Hz, 1H, CH_2Br), 3.09 (s, 1H, O*H*(Cl)), 3.03 (1H, s, O*H*(Br)), 1.64 (s, 3H, CH_3(Br)), 1.59 (s, 3H, CH_3(Cl)).

4.3. X-ray crystal structure determination for **4**

Suitable crystals of **4** were obtained by recrystallization from dichloromethane:hexane. Crystal data were collected using a Bruker SMART APEX CCD area detector diffractometer, and are listed in Table 1. A full sphere of reciprocal space was scanned by phi-omega scans. Pseudoempirical absorption correction based on redundant reflections was performed by the program SADABS [36]. The structures were solved by direct methods using SHELXS-973 [37] and refined by full-matrix least-squares on F^2 for all data using SHELXL-97 [38]. Hydrogen atoms attached to oxygen or nitrogen were located in the difference fourier map and allowed to refine freely with isotropic thermal displacement parameters. All other hydrogen atoms were added at calculated positions and refined using a riding model. Their isotropic thermal displacement parameters were fixed to 1.2 times (1.5 times for methyl groups) the equivalent one of the parent atom. Anisotropic thermal displacement parameters were used for all non-hydrogen atoms.

4.4. Biochemical experiments

4.4.1. Materials

DHT, and protamine sulfate were 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) for PC-3 and RPMI 1640 for LNCaP were purchased from Invitrogen. Fetal bovine serum, glutamine and kanamycine were obtained from Invitrogen. Prostate cancer cells LNCaP and PC-3 cells were from American Type Culture Collection (ATCC – LGC Promochem). [1,2-^{3}H]-DHT was purchased from NEN Life Science Product.

4.4.2. Determination of the RBA of the compounds for the androgen receptor (AR)

RBA values were measured on PanVera AR (750 pmol) purchased from Invitrogen. This AR is a recombinant rat protein expressed in *Escherichia coli*. The amino sequence of the ligand-binding domain of this AR is identical to that of the human AR LBD. 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 and 30 s 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} M 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 diameter glass microfibre filters GF/C filters using a Millipore 12 well filtration ramp. The filters were rinsed twice with cold phosphate buffer and then transferred into 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%.

4.4.3. 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 used [21]. 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 $\log P_{o/w} = 0.13418 + 0.98452 \log k_w'$.

4.4.4. Culture conditions

Cells were maintained in monolayer culture in DMEM or RPMI 1640 with phenol red/Glutamax I, supplemented with 9% of decomplemented fetal bovine serum and 0.9% kanamycine, at 37 °C in a 5% CO_2 air humidified incubator. For proliferation assays, PC-3 cells were seeded at a density of 15,000–25,000 cells per mL and 25,000–35,000 cells per mL for LNCaP in 24-well sterile plates in 1 mL of medium, supplemented with 9% of fetal bovine 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 (three wells for each product). After three days (D3), the incubation medium was removed and 2 mL of fresh medium containing the compounds was added. At different days (D3, D4, D5), 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.

Appendix A. Supplementary data

Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-785377. 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).

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