



Original article

Synthesis and anabolic/androgenic evaluation of novel 9 α -fluorosteroids

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ABSTRACT

3 β ,11 β -Dihydroxy-9 α -fluor-5 α -androstane-17-one (**2**), 3 β -acetoxy-9 α -fluor-11 β -hydroxy-5 α -androstane-17-one (**3**), 3 β -acetoxy-9 α -fluor-11 β ,17 β -dihydroxy-5 α -androstane (**4**), 3 β ,17 β -diacetoxy-9 α -fluor-11 β -hydroxy-5 α -androstane (**5**), 3 β -acetoxy-9 α -fluor-11 β -hydroxy-5 α -androstane 17 β -propionate (**6**), 3 β -acetoxy-9 α -fluor-11 β -hydroxy-5 α -androstane 17 β -enanthate (**7**), 3 β -acetoxy-9 α -fluor-11 β -hydroxy-5 α -androstane 17 β -isobutyrate (**8**) were synthesized in the present study. Compounds **2** and **8** exhibited higher anabolic activity than the rest of the synthesized compounds. The structure of all these newly synthesized compounds was confirmed by analytic spectral data (mass, ¹H NMR and ¹³C NMR).

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1. Introduction

Anabolic steroids cause retention of nitrogen, calcium, potassium, chloride, phosphate and water, as well as the growth of bones [1]. These drugs are used in the fast recovery from protein-wasting disorders. In HIV patients, anabolic steroids are used to regain lean muscle mass, as well as to prevent organ failure and secondary immune dysfunction [2]. These compounds have proven to be an effective oral therapy to promote weight gain after extensive surgery, chronic infections and severe trauma [3]. They are indicated in the treatment of anemia caused by deficient red-cell production, chronic obstructive pulmonary disease (attributed to emphysema as well as bronchitis) and metastatic cancer [4,5]. Many years ago the main goal of researchers in the anabolic steroid field was to synthesize a compound, which retained a high degree of anabolic activity coupled with a vastly diminished androgenic one. This property was quantified using the anabolic/androgenic ratio (A/A ratio). At present, the commercially available anabolic compounds were synthesized during the past 30 years of steroid's research. Some authors have said that it is not possible to even generalize what chemical modifications will reinforce the anabolic

activity with a simultaneous decrease in the androgenic activity [6,7].

On the other hand, it has been known that the substitution of a hydrogen atom(s) for a fluorine atom(s) in an organic compound can sometimes dramatically alter its physical, chemical, and biological properties [8]. Substitutions of 9 α -H by a halogen atom and 11 β -H by hydroxyl group have a positive effect on the anabolic/androgenic ratio [9], for example, fluoxymesterone presents 20 times the androgenic activity of the methyltestosterone [10–12].

In this paper we describe the synthesis, structural characterization and the anabolic/androgenic activity of new 9 α -fluorosteroids.

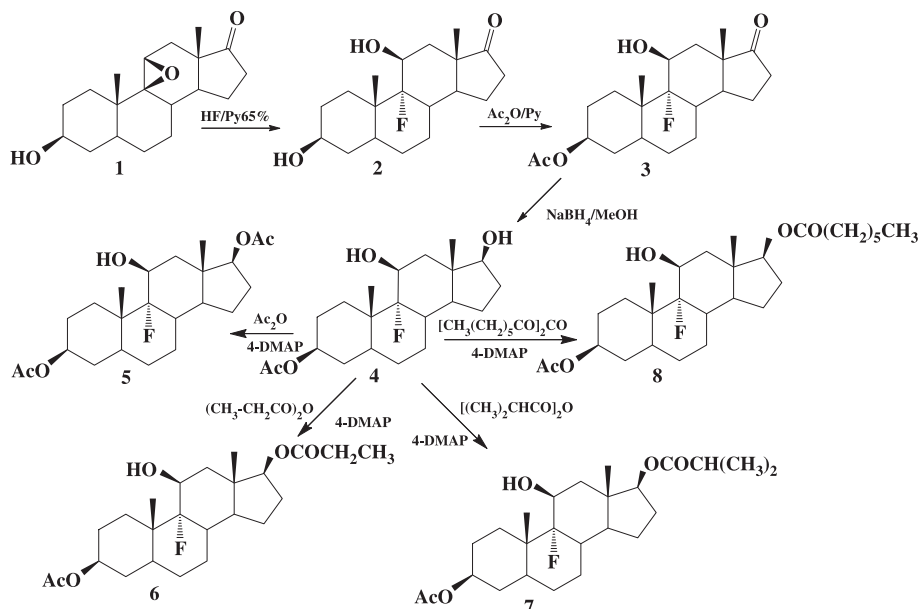
2. Chemistry

The present paper describes the preparation of several steroids containing fluorine with potential anabolic/androgenic activity as shown in Scheme 1.

The key intermediate, 9 β ,11 β -epoxy-5 α -androstan-3 β -ol-17-one (**1**) was synthesized from hecogenin according to a method described by Ruiz [13]. 3 β ,11 β -dihydroxy-9 α -fluor-5 α -androstane-17-one (**2**) was obtained by cleavage of epoxy **1** with HF/pyridine at 0 °C in chloroform. 3 β -Acetoxy-9 α -fluor-11 β -hydroxy-5 α -androstane-17-one (**3**) was prepared from **2** by acetylation in the presence of acetic anhydride with pyridine as catalyst. The reduction of C-17

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Scheme 1. Synthesis of fluorosteroid derivatives and their esters.

carbonyl group in compound **3** using sodium borohydride and methanol as solvent led to 5 α -androstane-3 β -acetoxy-11 β -17 β -dihydroxy-9 α -fluorine (**4**).

Esterification of compound **4** to obtain compounds **5–8** was made by the use of the respective acid anhydrides, 4-dimethylaminopyridine as catalyst and dichloromethane as solvent.

The structure elucidation of the products was supported by spectroscopic analysis and previous works. Since ^{19}F is a spin-1/2 nucleus, α -, β -, and some γ -carbon signals appear as doublets in the ^1H -BB-decoupled ^{13}C NMR spectra. $^nJ(^{13}\text{C}, ^{19}\text{F})$ data were useful in our work for the assignment of the ^{13}C chemical shift (Table 1). The observed results were in agreement with previously reported coupling constant values for similar compounds. The other signals were assignments using the data reported elsewhere [14–17].

3. Biological results and discussion

The pharmacological evaluation of the synthesized compounds was performed using the method of animal castration. This experimental model decreased the endogenous levels of testosterone causing atrophy of the genital organs and the skeletal muscle. The relative weight of the prostate (P), seminal vesicle (SV), and levator ani muscle (LA) was obtained for each tested product after 7 days of administration. In all cases our new products were compared with both an intact control (IC) and the orchidectomized control (OC). Testosterone was used as positive control because it is a drug reference with anabolic and/or androgenic activity. The experimental group administered with testosterone (T) showed significant statistical differences with regard to the group OC because it is able to recover physiologic hormonal levels and in some cases to surpass them.

Fig. 1 shows the evaluation of the anabolic activity of the synthesized compounds by measuring the weight of the levator ani muscle. It is observed that the effect of compound **2** at a dose of 40 mg/kg of weight differs statistically of group OC and it is similar to the group IC, recovering the physiologic hormonal levels.

The same behavior is presented for compounds **7** and **8** at doses of 20 and 40 mg/kg of weight, respectively.

Compounds **4** and **6** presented anabolic activity at doses of 20 and 40 mg/kg of weight. Compound **5** showed significant statistical difference at a dose of 20 mg/kg compared with the IC control group.

In Fig. 1 it is also observed that compound **3** differs statistically of the group IC in all doses showing that it does not present anabolic activity under the evaluated experimental conditions.

Fig. 2 shows the evaluation of the androgenic activity of the synthesized compounds by measuring the relative weight of the seminal vesicle (Fig. 2A) and the prostate (Fig. 2B). In Fig. 2A it is observed that compound **2** didn't present androgenic activity at doses of 10 and 20 mg/kg of weight and didn't differ statistically of the group OC but at a dose of 40 mg/kg of weight it differs statistically of the group OC as well as group IC (below 100%), although not reaching the physiologic hormonal levels, presenting a slight tendency toward the androgenic activity. This product (Fig. 2B) didn't present androgenic activity at prostate level to none of the rehearsed doses. Similarly, the enanthic ester **8** didn't also present androgenic activity in seminal vesicle and prostate to none of the tested doses.

Table 1
 ^{19}F , ^{13}C coupling constants (± 0.4 Hz), for α , β and γ carbons.

C (α , β , or γ)	Compounds						
	2 ^a	3 ^a	4	5	6	7	8
1 (γ)	2.9	2.2	2.3	2.5	2.3	2.3	2.2
5 (γ)	6.2	6.2	5.7	6.0	6.1	5.7	6.0
8 (β)	20.0	20.0	20.6	19.8	19.8	20.6	19.8
9 (α)	174	174.5	174.1	172.4	172.4	172.9	173.9
10 (β)	17.6	17.7	18.3	18.3	16.8	18.3	16.8
11 (β)	40.1	40.0	41.2	39.7	41.2	41.2	41.2
14 (γ)	2.4	2.3	2.5	2.5	2.4	2.2	2.3
19 (γ)	5.7	5.7	5.7	6.1	4.6	4.6	4.6

^a These coupling constants values were published by us previously [15].

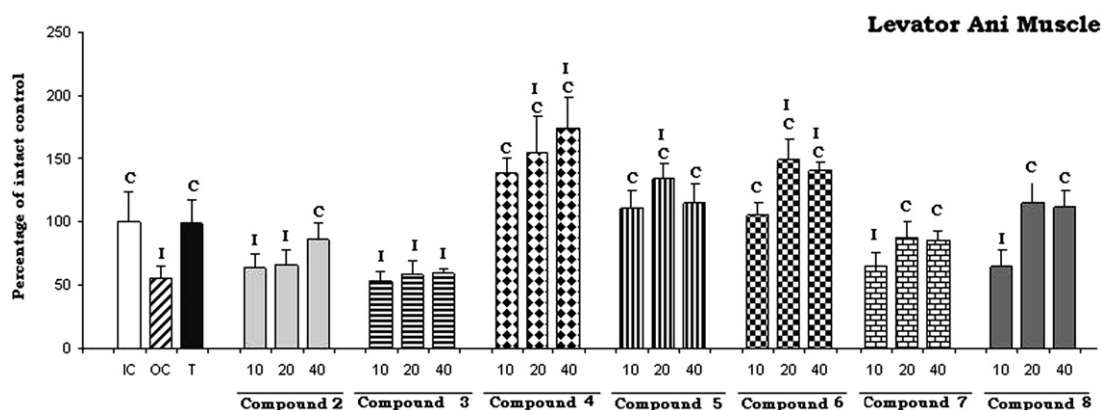


Fig. 1. Anabolic behavior of fluorine steroid esters to level of levator ani muscle; the evaluated groups for each product are represented as IC: intact control; OC: castrated control; T: testosterone (2 mg/kg of weight); 10, 20, 40: doses evaluated in mg/kg of corporal weight. The results represent the stocking standard deviation of each group. The letters C and I show statistical differences (Anova, $p \leq 0.05$) against the groups OC and IC, respectively.

In compounds **3** and **7** androgenic activity is not observed at level of seminal vesicle (Fig. 2A) in none of the rehearsed doses, however, at prostate level to all tested doses they present a slight tendency toward the androgenic activity (Fig. 2B).

Compounds **5** and **6** at the rehearsed doses show significant statistical differences against the group OC, as much in vesicle as in prostate (Fig. 2A and B). In the case of compound **6** at the dose of 40 mg/kg of weight is observed that it recovers and it overcomes the physiologic hormonal levels. Also, both products at the doses of 20 mg/kg of weight (**5**) and at 10 and 20 mg/kg of weight (**6**), recover and overcome the physiologic hormonal levels in the

prostate test (Fig. 2B). Therefore, compounds **5** and **6** present androgenic activity in both assays.

Compound **4** doesn't present androgenic activity in seminal vesicle at the dose of 10 mg/kg of weight. However, at doses of 20 and 40 mg/kg of weight it differs statistically from the OC group, as well as to the IC, not reaching physiologic hormonal levels. This compound presents a slight tendency toward the androgenic activity in the vesicle test at doses of 20 and 40 mg/kg of weight, however, in the rehearsed doses in the prostate test, it behaves as androgen, although at the dose of 40 mg/kg of weight it surpasses the physiologic hormonal

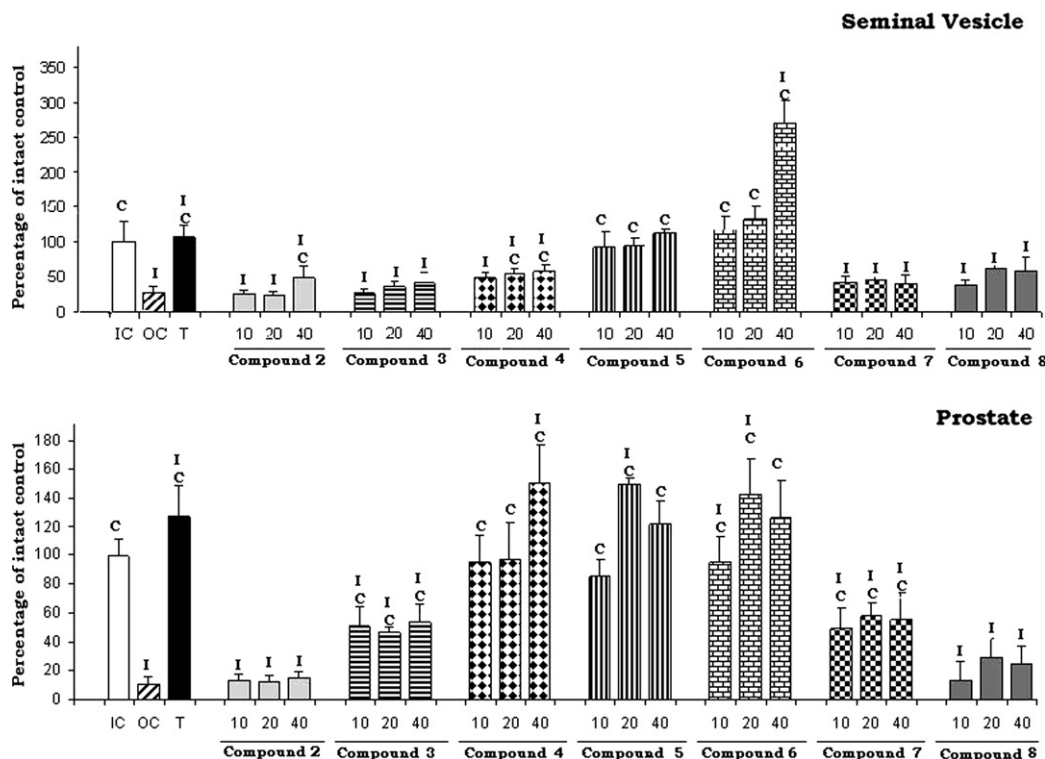


Fig. 2. Androgenic behavior of fluorine steroid esters to level of seminal vesicle and prostate; the evaluated groups for each product are represented as IC: intact control; OC: castrated control; T: testosterone (2 mg/kg of weight); 10, 20, 40: dose evaluated in mg/kg of corporal weight. The results represent the stocking standard deviation of each group. The letters C and I show statistical differences (Anova, $p \leq 0.05$) against the groups OC and IC respectively.

levels, since it presents significant differences regarding the groups OC and IC.

The obtained activity results of the anabolic and/or androgenic evaluation of the synthesized compounds (Figs. 1 and 2) could suggest that compounds **2** and **8** are good candidates of anabolic steroids with a minimum of androgenic activity.

4. Conclusion

Seven new fluorinated steroids were synthesized and spectroscopically characterized, 4 of them are esters.

Pharmacological results show that compound **2** and **8** present anabolic activity and almost null androgenic activity. These products could be considered as possible leader compounds.

5. Experimental protocols

5.1. General methods

Animals and treatment. Seventy-two immature (50–90 g) male Wistar rats were obtained from CENPALAB (Bejucal, Havana, Cuba) and acclimatized to the environmental conditions for 1 week before the tests. The cages were kept at $22 \pm 2^\circ\text{C}$, relative humidity 40–70%, and 12 h light/dark cycles with food and water ad libitum.

The orchidectomy was performed under pentobarbital (40 mg/kg) anesthesia. Animals were randomly chosen in ten groups. Intact control (IC): animals with physiological hormones levels (none castrated) and treated with sesame oil daily. Orchidectomized control (OC): castrated animals and treated with sesame oil injection daily. Testosterone propionate control (T): animals were treated with testosterone (2 mg/kg) daily. The other groups were treated with 10, 20, 40 mg/kg of compounds **2–8** during seven days. All the compounds evaluated and the testosterone propionate were dissolved in sesame oil and administrated by subcutaneous route. After 7 days of administration, all the animals were sacrificed and ventral prostate (P), seminal vesicles (SV), and levator ani muscle (LA) were carefully removed, released of adhering fat and immediately weighted. The relative weight of the P, SV, and LA were obtained for each product.

Statistical analysis. The weights of the organs removed were normalized to body weight of the each animal following this expression: Relative weight = organ weight/body weight and were converted to the percentage of the weights in the intact control group. Statistical analysis was performed using analysis of variance (ANOVA) to see significant differences between groups and then a Bonferroni's test $p \leq 0.05$ was established as the criterion of statistical significance.

5.2. Biological activity in vivo

The pharmacological evaluation of the synthesized products was performed using the method of animal castration [18,19]. This experimental model decreased the endogenous levels of testosterone causing atrophy of the genital organs and the skeletal muscle.

5.3. Preparation of the compounds

5.3.1. 3 β ,11 β -Dihydroxy-9 α -fluor-5 α -androstane-17-one (**2**)

5 g (16.4 mmol) of **1** dissolved in 75 mL of chloroform was added slowly to 25 mL of HF/pyridine solution cooled previously to 0°C . The progress of reaction was followed by TLC (chloroform–methanol, 9.6:0.4). The reaction was poured over a solution of K_2CO_3 10% (1500 mL), extracted with chloroform, washed with hydrochloric acid 6 N. After removing the solvent by evaporation in vacuum, the

crude product was crystallized from methanol to give **2**. (4.25 g, 80%), m.p. 208–210 $^\circ\text{C}$. ^1H NMR 250 MHz (CDCl_3 , ppm): 1.01 (3H, s, $\text{H}_3\text{C}-18$); 1.09 (3H, s, $\text{H}_3\text{C}-19$); 3.5 (1H, m, H-3); 4.13 (1H, dt, H-11); $^3J_{(\text{H}11\text{ec},\text{H}12\text{ax})} = ^3J_{(\text{H}11\text{ec},\text{H}12\text{ec})} = 2.7$ Hz, $^3J_{(\text{H}11,\text{F})} = 9.1$ Hz).

^{13}C NMR (CDCl_3 , ppm): **C17** 220.9; **C9** 99.6; **C3** 70.1; **C11** 69.5; **C13** 46.5; **C14** 45.3; **C10** 39.7; **C12** 36.9; **C5** 36.8; **C4** 36.6; **C16** 35.4; **C8** 33.5; **C2** 30.4; **C1** 29.7; **C6** 27.0; **C7** 25.2; **C15** 21.3; **C19** 15.4; **C18** 15.1. MS m/z : 324 (M^+).

5.3.2. 3 β -Acetoxy-9 α -fluor-11 β -hydroxy-5 α -androstane-17-one (**3**)

1 g (3.1 mmol) of compound **2** was added to a stirred mixture of 5 mL of acetic anhydride (5.4 g; 52.9 mmol) and 1 mL (0.983 g; 12.4 mmol) of pyridine. The reaction was agitated during 18 h. The progress of reaction was followed by TLC (toluene–ethyl acetate, 2:1). The reaction was poured over 50 mL of water and 30 g of ice. The crude product was filtered, washed with water, dried and crystallized from methanol to give **3**. (1 g, 88%), m.p. 236–238 $^\circ\text{C}$. ^1H NMR 250 MHz (CDCl_3 , ppm): 1.09 (3H, s, $\text{H}_3\text{C}-18$); 1.17 (3H, s, $\text{H}_3\text{C}-19$); 2.01 (3H, s, $\text{H}_3\text{C}-21$); 4.24 (1H, dt, H-11); $^3J_{(\text{H}11\text{ec},\text{H}12\text{ax})} = ^3J_{(\text{H}11\text{ec},\text{H}12\text{ec})} = 2.7$ Hz, $^3J_{(\text{H}11,\text{F})} = 9.1$ Hz); 4.66 (1H, m, H-3). ^{13}C NMR (CDCl_3 , ppm): **C17** 218.9; **C20** 170.6; **C9** 99.4; **C3** 72.9; **C11** 70.2; **C13** 46.3; **C14** 45.3; **C10** 39.8; **C12** 37.3; **C5** 36.8; **C16** 35.3; **C8** 33.7; **C4** 33.2; **C1** 29.9; **C6** 27.0; **C2** 26.9; **C7** 25.1; **C15** 21.4; **C21** 21.3; **C19** 15.6; **C18** 15.3. MS m/z : 366 (M^+).

5.3.3. 3 β -Acetoxy-9 α -fluor-11 β ,17 β -dihydroxy-5 α -androstane (**4**)

0.93 g sodium borohydride (24.5 mmol) was added to a solution of **3** (4.5 g, 12.2 mmol) in methanol (45 mL) and the reaction mixture was agitated vigorously at room temperature for 30 min. The progress of reaction was followed by TLC (toluene–ethyl acetate, 2:1). The reaction was worked up and the solid crystallized from methanol to give **4**. (4.5 g, 100%, m.p. 162–164 $^\circ\text{C}$). ^1H NMR 400 MHz (CDCl_3 , ppm): 0.98 (3H, s, $\text{H}_3\text{C}-18$); 1.16 (3H, s, $\text{H}_3\text{C}-19$); 2.02 (3H, s, $\text{H}_3\text{C}-21$); 3.67 (1H, dd, H-17); $^3J_{(\text{cisH}16,\text{H}17)} = 8.93$ Hz, $^3J_{(\text{transH}16,\text{H}17)} = 7.55$ Hz); 4.15 (1H, dt, H-11); $^3J_{\text{H}11,\text{H}12} = 2.65$ Hz, $^3J_{\text{H}11,\text{F}} = 9.1$ Hz); 4.66 (1H, m, H-3). ^{13}C NMR (CDCl_3 , ppm): **C20** 170.5; **C9** 99.3; **C17** 81.7; **C3** 73.0; **C11** 70.1; **C14** 44.8; **C12** 42.3; **C13** 41.7; **C10** 39.7; **C5** 36.9; **C8** 34.1; **C4** 33.3; **C16** 30.3; **C1** 29.9; **C6**, 27.2; **C2** 27.0; **C7** 25.8; **C15** 23.3; **C21** 21.5; **C19** 15.7; **C18** 13.3. MS m/z : 368 (M^+).

5.3.4. 3 β ,17 β -Diacetoxy-9 α -fluor-11 β -hydroxy-5 α -androstane (**5**)

0.66 g of 4-dimethylaminopyridine (5.4 mmol) and 1 mL of acetic anhydride (1.08 g; 10.5 mmol) were added to a solution of **4** (1 g, 2.72 mmol) in dichloromethane (50 mL). The reaction mixture was agitated vigorously at room temperature for 1 h. The progress of reaction was followed by TLC (cyclohexane–ethyl acetate, 2:1). The reaction mixture was placed in a separator funnel and washed first with hydrochloric acid 6 N, later with 10% sodium bicarbonate solution and finally with water until neutrality. The organic solvent was removed by evaporation in vacuum and the crude solid crystallized from methanol to give **5**. (0.92 g, 83%, m.p. 192–194 $^\circ\text{C}$). ^1H NMR 300 MHz (CDCl_3 , ppm): 1.01 (3H, s, $\text{H}_3\text{C}-18$); 1.16 (3H, s, $\text{H}_3\text{C}-19$); 2.02 (3H, s, $\text{H}_3\text{C}-21$); 2.04 (3H, s, $\text{H}_3\text{C}-23$); 4.16 (1H, dt, H-11); $^3J_{(\text{H}11\text{ec},\text{H}12\text{ax})} = ^3J_{(\text{H}11\text{ec},\text{H}12\text{ec})} = 2.6$ Hz, $^3J_{(\text{H}11,\text{F})} = 9.3$ Hz); 4.60 (1H, dd, H-17); $^3J_{(\text{cisH}16,\text{H}17)} = 9.0$ Hz $^3J_{(\text{transH}16,\text{H}17)} = 7.2$ Hz); 4.68 (1H, m, H-3). ^{13}C NMR (CDCl_3 , ppm): **C22** 170.9; **C20** 170.4; **C9** 99.1; **C17** 82.8; **C3** 72.9; **C11** 70.0; **C14** 44.7; **C12** 42.6; **C13** 41.5; **C10** 39.8; **C5** 36.8; **C8** 33.9; **C4** 33.3; **C1** 29.9; **C16** 27.3; **C6** 27.2; **C2** 26.9; **C7** 25.8; **C15** 23.4; **C21** 21.5; **C23** 21.2; **C19** 15.7; **C18** 14.3. MS m/z : 410 (M^+).

5.3.5. 3 β -Acetoxy-9 α -fluor-11 β -hydroxy-5 α -androstane 17 β -propionate (**6**)

0.66 g of 4-dimethylaminopyridine (5.4 mmol) and 0.91 mL of propionic anhydride (0.92 g; 7.0 mmol) were added to a solution of

4 (1 g, 2.72 mmol) in dichloromethane (50 mL). The reaction mixture was agitated vigorously at room temperature for 1 h. The progress of reaction was followed by TLC (cyclohexane–ethyl acetate, 2:1). The reaction mixture was treated in the same way that **5** and **6** were obtained in pure form. (0.89 g, 77%, m.p. 177–178 °C). ¹H NMR 300 MHz (CDCl₃, ppm): 1.01 (3H, s, H₃C-18); 1.13 (3H, t, H₃C-24), *J*(H₂₃,H₂₄) = 7.6 Hz; 1.16 (3H, s, H₃C-19); 2.02 (3H, s, H₃C-21); 2.32 (2H, q, H-23), *J*(H₂₃,H₂₄) = 7.6 Hz; 4.14 (1H, dt, H-11 ³*J*(H₁₁ec,H₁₂ax) = ³*J*(H₁₁ec,H₁₂ec) = 2.4 Hz, ³*J*(H₁₁,F) = 6.8 Hz); 4.61 (1H, dd, H-17 ³*J*(cisH₁₆,H₁₇) = 9.2 Hz ³*J*(transH₁₆,H₁₇) = 7.2 Hz); 4.66 (1H, m, H-3).

¹³C NMR (CDCl₃, ppm): C22 174.3; **C20** 170.4; **C9** 99.9; **C17** 82.7; **C3** 72.3; **C11** 70.0; **C14** 44.6; **C12** 42.5; **C13** 41.5; **C10** 39.7; **C5** 36.8; **C8** 33.7; **C4** 33.2; **C1** 29.8; **C23** 27.8; **C6** 27.4; **C16** 27.3; **C2** 26.9; **C7** 25.8; **C15** 23.3; **C21** 21.1; **C19** 15.6; **C18** 14.2; **C24** 9.3. MS *m/z*: 424 (M⁺).

5.3.6. 3β-Acetoxy-9α-fluor-11β-hydroxy-5α-androstane 17β-isobutyrate (**7**)

0.80 g of 4-dimethylaminopyridine (6.55 mmol) and 2.07 mL of isobutyric anhydride (1.97 g; 12.5 mmol) were added to a solution of **4** (2 g, 5.4 mmol) in dichloromethane (10 mL). The reaction mixture was agitated vigorously at room temperature for 1 h. The progress of reaction was followed by TLC (cyclohexane–ethyl acetate, 2:1). The reaction mixture was treated in the same way that **5** and **7** were obtained in pure form. (1.99 g, 84%), m.p. 136–140 °C. ¹H NMR 300 MHz (CDCl₃, ppm): 1.03 (3H, s, H₃C-18); 1.15 (3H, d, H₃C-24, *J*(H₂₄,H₂₃) = 6.9 Hz); 1.17 (3H, d, H₃C-25 ³*J*(H₂₅,H₂₃) = 6.9 Hz); 1.31 (3H, s, H₃C-19); 2.02 (3H, s, H₃C-21), 2.53 (1H, m, H-23); 4.36 (1H, bs, H-11); 4.61 (1H, dd, H-17 ³*J*(cisH₁₆,H₁₇) = 9.6 Hz ³*J*(transH₁₆,H₁₇) = 7.5 Hz); 4.65 (1H, m, H-3). ¹³C NMR (CDCl₃, ppm): C22 174.0; **C20** 170.4; **C9** 99.0; **C17** 82.4; **C3** 72.9; **C11** 70.1; **C14** 44.7; **C12** 42.6; **C13** 41.6; **C10** 39.6; **C5** 36.8; **C8** 33.8; **C23** 34.2; **C4** 33.2; **C1** 29.6; **C16** 27.1; **C6** 27.3; **C2** 26.8; **C7** 25.7; **C15** 23.3; **C21** 21.4; **C25** 19.1; **C24** 19.0; **C19** 15.5; **C18** 14.2. MS *m/z*: 438 (M⁺).

5.3.7. 3β-Acetoxy-9α-fluor-11β-hydroxy-5α-androstane 17β-enanthate (**8**)

0.66 g of 4-dimethylaminopyridine (5.4 mmol) and 1.88 mL of enanthic anhydride (1.72 g; 7.1 mmol) were added to a solution of **4** (1 g, 2.72 mmol) in dichloromethane (50 mL). The reaction mixture was agitated at room temperature for 1 h. The progress of reaction was followed by TLC (cyclohexane–ethyl acetate, 2:1). The reaction mixture was treated in the same way that **5** and **8** were obtained in pure form. (0.94 g, 72%), m.p. 119–120 °C. ¹H NMR 400 MHz (CDCl₃, ppm): 0.88 (3H, t, H₃C-28 ³*J*(H₂₇,H₂₈) = 6.8); 1.01 (3H, s, H₃C-18); 1.16 (3H, s, H₃C-19); 2.02 (3H, s, H₃C-21); 2.29 (2H, t, H₂C-23 ³*J*(H₂₃,H₂₄) = 7.6); 4.14 (1H, dt, H-11 ³*J*(H-11, H-12ax) = ³*J*(H-11,H-12ec) =

2.8 Hz, ³*J*(H,F) = 9.2 Hz); 4.61 (1H, dd, H-17 ³*J*(cisH₁₆,H₁₇) = 9.2 Hz ³*J*(transH₁₆,H₁₇) = 7.2 Hz); 4.66 (1H, m, H-3). ¹³C NMR (CDCl₃, ppm): C22 173.6; **C20** 170.4; **C9** 99.0; **C17** 82.5; **C3** 72.9; **C11** 70.1; **C14** 44.7; **C12** 42.7; **C13** 41.6; **C10** 39.7; **C5** 36.9; **C23** 34.6; **C8** 33.9; **C4** 33.3; **C24** 31.5; **C1** 29.9; **C25** 28.9; **C16** 27.4; **C6** 27.2; **C2** 26.9; **C7** 25.8; **C26** 25.1; **C15** 23.4; **C27** 22.6; **C21** 21.5; **C19** 15.7; **C18** 14.4; **C28** 14.2. MS *m/z*: 480 (M⁺).

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