Antiandrogenic Effect of 16-Substituted, Non-substituted and D-Homopregnane Derivatives

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The pharmacological activities of 12 pregnane derivatives (4—15) were determined on gonadectomized male hamster flank organs and seminal vesicles as antiandrogens and as 5α-reductase inhibitors. The results from this study indicate that subcutaneous injection of testosterone for 3 d increased the diameter of the pigmented spot in the flank organs, whereas finasteride when injected with testosterone decreased the size of the spot significantly when steroids 4—15 were injected together with testosterone, the diameter of the flank organs of gonadectomized male hamsters, decreased significantly ($p<0.005$) compared to testosterone. Compound 11 was the most active steroid and reduced the diameter of the pigmented spot more than the other synthesized steroids or finasteride. Subcutaneous injections of testosterone to gonadectomized animals restore the seminal vesicle size lost upon castration. Injection of testosterone plus finasteride decreased significantly the weight of these glands ($p<0.005$). Steroids 5—15 when injected with testosterone decreased the weight of the seminal vesicles compared to testosterone. Finasteride is a good inhibitor of the conversion of testosterone to dihydrotestosterone (DHT) (low formation of DHT) measured as pmole of DHT/g of protein/h. Steroids 6—15 inhibited the conversion of testosterone to DHT as compared to testosterone however finasteride and 10 appeared to be the most effective compounds. Castration increases the protein content of the seminal vesicles (control) expressed as μg/mg of tissues. Testosterone tends to decrease it significantly, as did compounds 4, 5, 7, 9, and 15. We demonstrated that DHT as well as cyproterone acetate and steroids 5, 6, 8, 9, 11, and 14 at increasing non radioactive steroid concentration, inhibited the binding of [^{3}H]DHT to cytosolic androgen receptor (AR), as indicated by its K_i values. However, 4, 7, 10, 12, and 13 did not have any inhibitory effect.

Key words seminal vesicle; 5α-reductase; testosterone conversion; D-homo steroid

Antiandrogens offer a potentially useful treatment for androgen mediated diseases such as prostate cancer, benign prostatic hyperplasia, seborrhea, androgenic alopecia, acne and precocious puberty.[1] The Health Secretary[2] reports that prostate carcinoma is an important cause of death in Mexico. The mortality rate per 100000 is about 72.2 for aged men. The potential use of antiandrogens in the treatment of prostate cancer represents an alternative to prostatic operation, which is associated with several side effects and is probably the surgery men fear most.[3]

It has been reported that progesterone (P_4) and deoxycorticosterone inhibit dihydrotestosterone (DHT) (**2**) formation by competing with the Δ^4-3-keto site of testosterone (T) (**1**) for the 5α-reductase enzyme.[4] Previous studies carried out in our laboratory demonstrated that a bromine atom at the C-6 position of the P_4 skeleton increases the inhibitory power of the P_4 molecule on the conversion of T to DHT.[5] Furthermore, a C-17 benzoyloxy moiety in a 4-bromoprogesterone skeleton also contributes to the antiandrogenic activity as measured by reduction in the weight of seminal vesicles, and by the amount of produced DHT. These steroids also showed a much higher inhibitory activity on the conversion of T to DHT than the presently used finasteride (**3**).[6]

Compound **3** is a steroid that has successfully been used for the treatment of benign and malign prostatic hyperplasia.[7] This compound inhibits the activity of 5α-reductase enzyme type 2 that is present in the prostate, and has also a limited inhibitory effect on type 1 enzyme found in the skin.[8]

In this study, we report the antiandrogenic activity of 12 different steroidal compounds **4**—**15** that were synthesized in our laboratory. The antiandrogenic activity was determined in flank organs, seminal vesicles and also *in vitro* by measuring the amount of DHT expressed as pmole of DHT/g of protein/h. In addition, these compounds were evaluated as antagonists for the androgen receptor (AR).

Flank organs are two pigmented nodules located in the dorsal skin surface of hamsters. In female hamsters, the diameter of the pigmented spot measures 2 mm, whereas in males it is 8 mm. In males, these nodules shrink upon castration and they resemble the nodules of the females.[9] However, daily injections of T (**1**) or 5α-DHT (**2**) restore their original size.[10,11] Many steroidal and non-steroidal compounds have been evaluated as antiandrogens using flank organ as a model.[12,13]

Seminal vesicles are male accessory organs that are also androgen-dependent. These organs are capable of reducing T to DHT in both intact and gonadectomized animals and have also been used for evaluation of steroidal and non-steroidal compounds as antiandrogens.[14]

In this study, we evaluated the following compounds: 20-ethylenedioxy-16β-phenylpregn-5-ene-3β,17α-diol (**4**) (Fig. 1); 3β,17α-dihydroxy-17β-methyl-16β-phenyl-D-homopregn-5-en-17a-one (**5**); 17α-hydroxy-17β-methyl-16β-phenyl-D-homopregna-4,6-diene-3,17a-dione (**6**); 17α-acetoxy-17β-methyl-16β-phenyl-D-homopregna-4,6-diene-3,17a-dione (**7**); 17α-hydroxy-16β-methylpregna-1,4,6-triene-3,20-dione (**8**); 16β-methyl-17α-propionyloxypregna-1,4,6-trienediene-3,20-dione (**9**); 17α-acetoxy-16β-methylpregna-1,4,6-triene-3,20-dione (**10**); 17α-acetoxy-4-bromopregna-1,4-diene-3,20-dione (**11**); 3β-acetoxypregna-5,16-dien-20-one (**12**); 3β-hydroxy-16α,17α-epoxypregn-5-ene-20-one (**13**); 3β-

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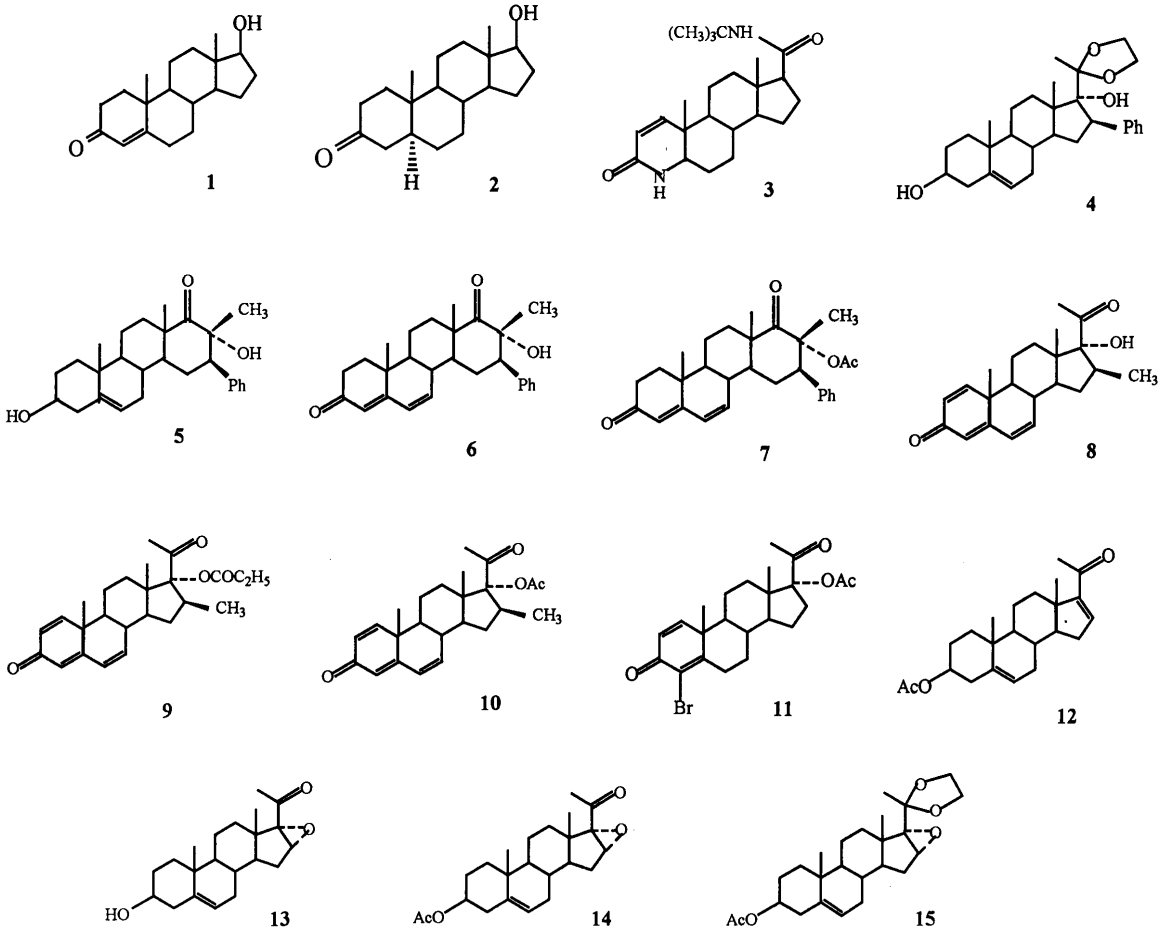

Fig. 1. Steroidal Antiandrogens

acetoxy-16α,17α-epoxypregn-5-ene-20-one (**14**) and 16α,17α-epoxy-20-ethylenedioxypregn-5-ene-3β-yl acetate (**15**).

Methods and Results

Effect of Synthesized Steroids on Flank Organs, Seminal Vesicles, DHT Formation and Protein Synthesis

Flank Organ Test After castration, the diameter of the pigmented spot on the flank organs (control) decreased significantly ($p<0.05$) compared to that of the uncastrated animals. Subcutaneous injections of vehicle alone did not change the size of the organs, however treatment with T (**1**) restored the diameter of the pigmented spot (Table 1). When T (**1**) and **3** were injected together, the diameter of the pigmented spot on the flank organs decreased significantly (2 mm) as compared to that of the gonadectomized animals treated with T (**1**) (8 mm). All synthesized steroids **4**—**15** when injected with T (**1**) decreased the diameter of the spot as compared to the control T. The most effective compounds in this study were steroids **6** and **11**, which reduced the diameter of the flank organs to 2.0 and 1.5 mm respectively. This data suggests that steroids **6** and **11** have similar inhibitory activity towards the enzyme 5α-reductase as compared to the standard **3**.

Seminal Vesicles After castration, the weight of the male hamster seminal vesicles decreased significantly ($p<0.05$) (control) compared to that of the normal glands. Treatment with vehicle alone did not change this condition (Table 1) whereas subcutaneous injections of 200 μg of T (**1**) (T) for 3 d significantly increased ($p<0.05$) the weight of the seminal vesicles in castrated male hamsters.

When T (**1**) and **3** were injected together, the weight of the seminal vesicles decreased significantly ($p<0.005$) as compared to the experiment in which the animals were treated with T only (Table 1).

The injection of steroids **5**—**15** together with T (**1**), decreased ($p<0.005$) the weight of the seminal vesicles as

Table 1. The Diameter of Flank Organs, and the Weight and Protein Content of Seminal Vesicles Were Measured in Animals Which Received Subcutaneous Treatment of the Synthesized Steroids±Standard Deviations

Treatment	Diameter of flank organs (mm)	Weight of seminal vesicles (mg)	Protein content (μg/mg of tissue)
Control	2.0±0.00	91.5±13.8	50.99±4.0
T	8.0±0.00	382.4±10.8	28.10±4.2
T+**3**	2.0±0.02	226.2±30	41.20±9.2
T+**4**	5.5±0.50	350±50	25.00±5
T+**5**	2.5±0.50	173±26	22.82±1.1
T+**6**	2.0±0.00	127±14	37.62±1.1
T+**7**	3.5±0.50	214.3±13.4	22.40±1.9
T+**8**	2.5±0.50	154±31	35.79±1.4
T+**9**	3.0±0.00	120±10	18.00±5.0
T+**10**	3.0±0.00	121.5±23	35.97±1.4
T+**11**	1.5±0.70	111.6±5.4	41.33±1.4
T+**12**	3.0±0.00	111.4±1	52.18±2.0
T+**13**	3.0±0.00	186.3±19	59.18±8.6
T+**14**	2.5±0.60	199.5±24	56.58±2.6
T+**15**	4.0±0.00	179.2±4	25.22±1.0

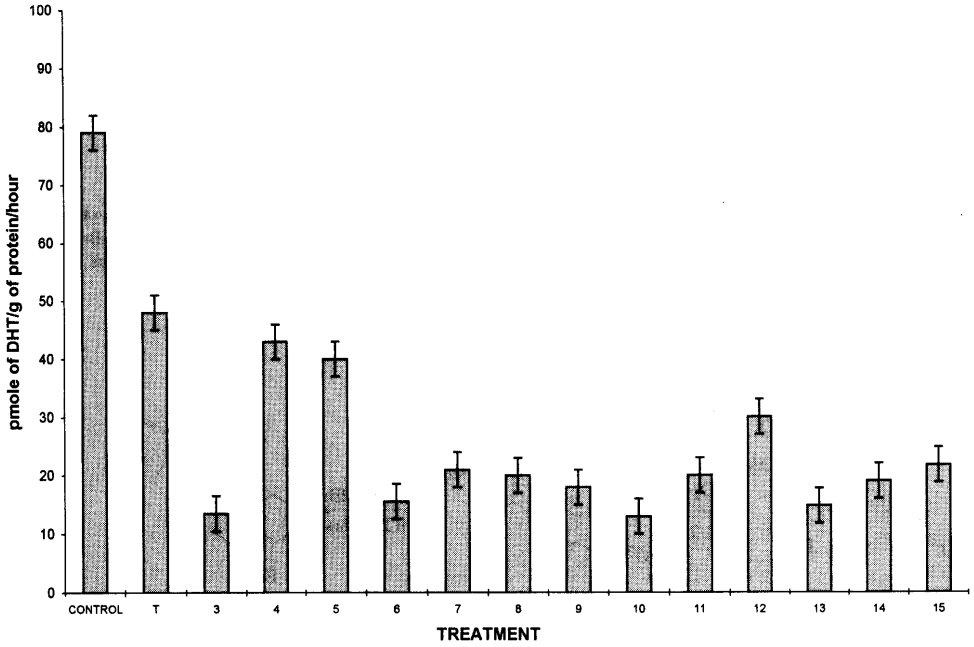

Fig. 2. The Effect of the Synthesized Steroidal Antiandrogens on T to DHT Conversion in Seminal Vesicles Expressed as pmol of DHT/g of Protein/h± Standard Deviation

compared to the T and **4** treated animals. The most effective compounds for decreasing the weight of the seminal vesicles were steroids **6**, **9**—**12**.

Conversion of T to DHT The extracts from castrated male hamster seminal vesicles were subjected to TLC analysis. The zone corresponding to the DHT (**2**) standard (*Rf* value 0.34) of each experimental chromatogram was eluted and the radioactivity determined.

The results from Fig. 2 obtained from two separate experiments performed in duplicate showed that in the seminal vesicles of control animals, a significant conversion of T to DHT took place.

Figure 2 shows the significant difference ($p<0.05$) in the conversion of [^{3}H]T (**1**) to [^{3}H]DHT (**2**) between the control animals treated with T and those treated with T plus **3**. The data from this study clearly indicate that **3** is a good inhibitor of the conversion of T (**1**) to DHT (**2**) at pH 6.

The effect of different steroidal structures **4**—**15** on the rate of DHT formation expressed as pmoles of DHT/g of protein/h is shown in Fig. 2. Compounds **4** and **5** showed a higher *in vitro* conversion of T to DHT ($p<0.05$) than the other synthesized steroids. On the other hand, **6**—**15** exhibited a lower DHT conversion than the control and T-treated animals. Compounds **6**, **10** and **13** demonstrated the same inhibitory effect as **3**.

Antagonistic Activity of the Synthesized Steroids for ARs The effect of increasing non-radioactive natural and synthetic steroid concentrations upon [^{3}H]DHT binding to ARs from seminal vesicles are presented in Fig. 5. The K_i for the synthesized steroids showed the following order of affinity to ARs : DHT>CA>8>11>5>9>14>6 (see Table 2).

Protein Content The protein content was determined using the well known Bradford dye-binding method.[15]

In seminal vesicles the protein content under different treatments is shown in Table 1. Castration tends to increase the protein content (control) expressed as μg/mg of tissue whereas T (**1**) decreased it, as previously reported by our group.[5,6]

Table 2. Inhibition Constants (K_i) of Different Steroids

Steroid	K_i
DHT	10.0
CA	10.8
P_4	NA
4	NA
5	17.2
6	41.0
7	NA
8	16.0
9	19.0
10	NA
11	16.5
12	NA
13	NA
14	22.0

Results are given in [nM]. NA; no affinity for androgen receptors.

Discussion

This study reports the antiandrogenic effect of a variety of 16-phenylsubstituted-D-homo compounds **5**—**7**, 16-methyl-substituted steroids **8**—**10**, a 4-bromo compound **11** without a methyl group at C-16 and the epoxy compounds **13**—**15**.

Compounds **5**—**10**, **13**—**15** were prepared from the commercially available 16-dehydropregnenolone acetate (**12**). Epoxidation of the double bond at C-16 in **12** with hydrogen peroxide and sodium hydroxide afforded the epoxy derivative **13** (Fig. 4). Acetylation of **13** in the usual manner yielded the acetoxy compound **14**. Protection of the carbonyl group in **14** was effected with ethylene glycol, trimethyl orthoformate and *p*-toluenesulfonic acid. The resulting dioxolane derivative **15** was treated with phenylmagnesium bromide in tetrahydrofuran (THF) at reflux to give C-16 phenyl derivative **4** (Fig. 3). Hydrolysis of the dioxolane ring of **4** to recover the carbonyl moiety was carried out with perchloric

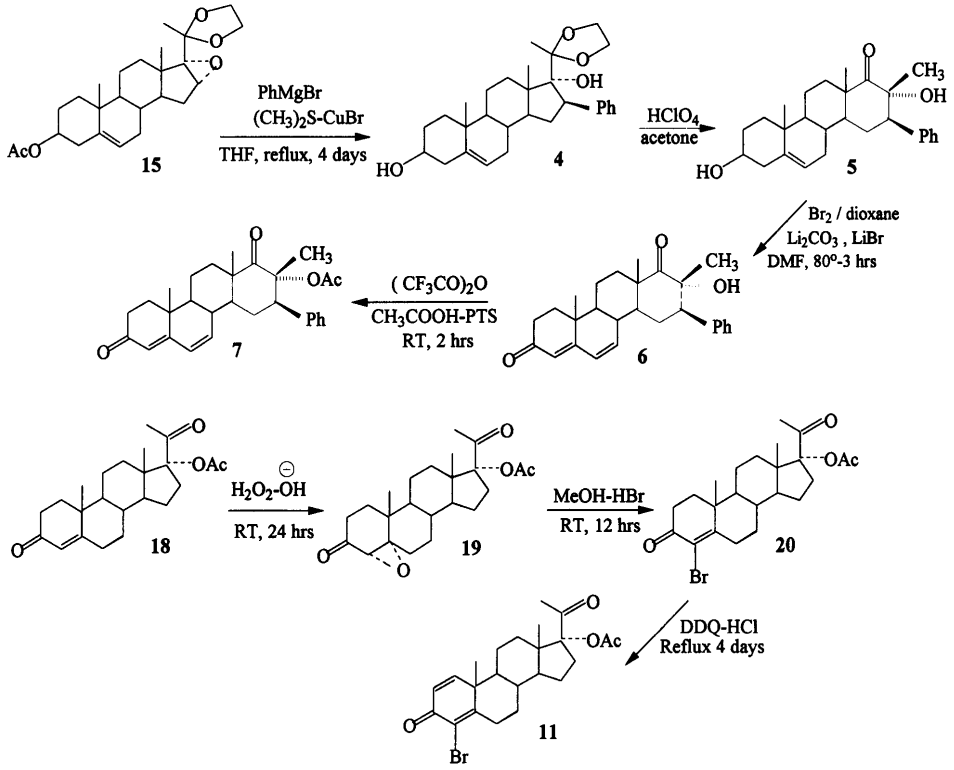

Fig. 3. Synthesis of the Steroidal Compounds from the Intermediate 16α,17α-Epoxy-20-ethylenedioxypregn-5-en-3β-yl Acetate

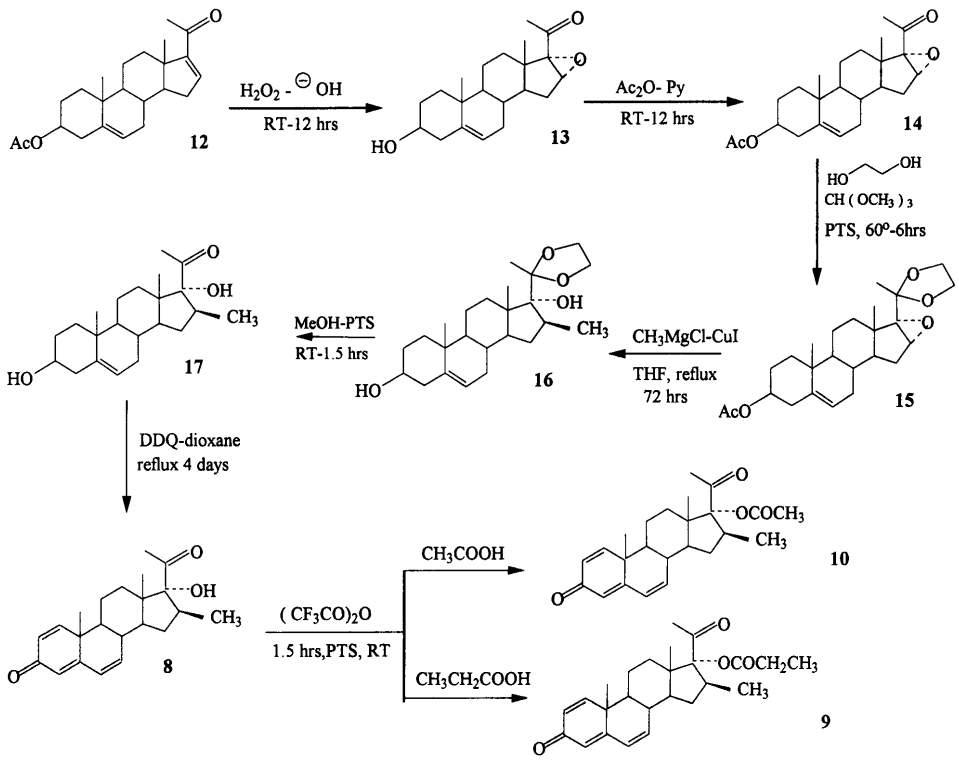

Fig. 4. Synthesis of the Steroidal Compounds from the Commercially Available 16-Dehydropregnenolone Acetate

acid in acetone. In this reaction, expansion of the D-ring took place thus forming D-homosteroid **5**.[16] Treatment of **5** with lithium carbonate, lithium bromide and bromine in *N*,*N*-dimethylformamide afforded the 4,6-diene-3-one moiety **6**. Acetylation of the hydroxyl group in **6** with trifluoroacetic anhydride and acetic acid yielded the desired acetoxy derivative **7**.

Compounds **8**—**10** were synthesized from the intermediate **15** (Fig. 4). Reaction of **15** with methylmagnesium chloride afforded the 16β-methyl derivative **16**. Treatment of **16** with methanol and *p*-toluenesulfonic acid yielded the C-20 carbonyl derivative **17** (ref. 3 describes the synthesis of these compounds). Upon reflux with DDQ (dichlorodicyanobenzoquinone), **17** afforded the steroid **8**. Esterification of **8** with

acetic acid–trifluoroacetic anhydride yielded the desired acetoxy derivative **10**. On the other hand, treatment of **8** with propionic acid–trifluoroacetic anhydride afforded the propionyloxy derivative **9**.

The 4-bromo derivative **11** (Fig. 3) was synthesized from the commercially available 17α-acetoxyprogesterone (**18**). Epoxidation of the C-4 double bond in **18** afforded the 4,5-epoxy compound **19**. Opening of the oxirane ring in **19** was carried out with methanol and hydrogen bromide. The resulting 4-bromo derivative **20** (described in ref. 6) was treated with DDQ in dioxane to afford desired compound **11**.

As can be seen from Table 1 (diameter of flank organs), steroid **6** has the same inhibitory effect as the standard compound **3**, whereas **11** has a higher inhibitory activity for the enzyme 5α-reductase than commercially available **3**. This compound has a bromine atom at C-4 and is very similar to compounds having a bromine atom at C-6 previously synthesized in our laboratory, which also showed potent inhibitory activity towards 5α-reductase, as well as antiandrogenic activity.[5] Flank organs have the capacity to convert T to DHT[11] and in the skin[8] the 5α-reductase enzyme type 1 is more abundant than type 2. Compound **3** is a typical inhibitor for type 2 5α-reductase enzyme, however it can also inhibit the type 1 enzyme albeit to a smaller degree. In our study, all compounds (**4**—**15**) reduced the size of the pigmented spot as compared to T; (diameter 8 mm), however this does not exclude the presence of type 2 enzyme in this tissue. These data also suggest that compounds **4**—**15** could also inhibit the FAR-17A gene.[17]

Table 1 shows the antiandrogenic effect of steroids **4**—**15** related to the weight of the seminal vesicles of castrated male hamsters treated with T (**1**) or a combination of T (**1**) with the synthesized steroids. These data clearly indicate that compounds **5**—**15** showed a higher antiandrogenic effect (lower weight of the seminal vesicles) than the finasteride treated animals. These effects are not only the result of inhibition of conversion of T to DHT[18]; but could also involve inhibition of the synthesis and/or release of pituitary gonadotropins.

Figure 2 shows the conversion of [^{3}H]T to [^{3}H]DHT expressed as pmole of protein per hour. In the control animals, conversion of T to DHT was higher than in the T (**1**) treated animals. This can be explained since the control animals were treated with vehicle only, whereas in all other experiments, they were treated with T (**1**) and the synthesized steroid, which could dilute the radiolabeled marker in the seminal vesicles. It is also possible that the 5α-reductase enzyme was synthesized in a higher amount in the control as compared to the T treated animals.

Compound **3** reduces the conversion of T to DHT substantially and therefore can be considered a good inhibitor of the *in vitro* T to DHT conversion at pH 6 (Fig. 2). These results are in agreement with those obtained by other authors, who reported the inhibitory effect of finasteride and similar compounds on 5α-reductase type 2-activity.[8] All synthesized steroids, with the exception of **4** and **5**, inhibited the conversion of T to DHT at pH 6 in seminal vesicles homogenates from castrated male hamsters treated with a dose of 200 μg as compared to T (**1**). Compounds **10** and **3** showed similar inhibition of the T to DHT conversion.

Compound **5** decreased the weight of the seminal vesicles, when compared to the T treated animals (Table 1), however it did not show a similar effect in the T to DHT conversion (Fig. 2). These data suggest that **5** is probably an agonist of the AR.

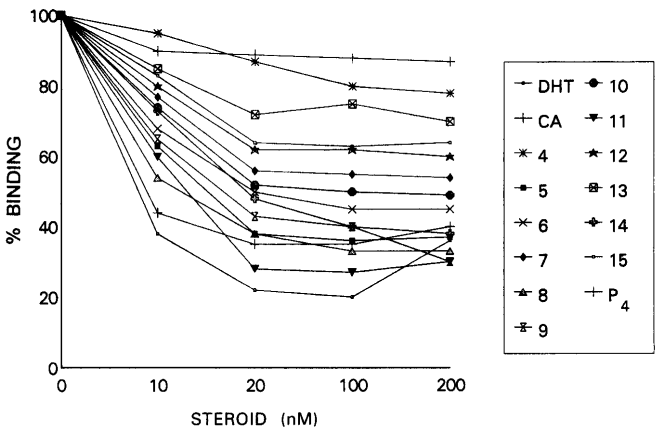

Fig. 5. Binding Specificity

Gonadectomized male hamster seminal vesicle cytosol incubated in the presence of 1 nM [^{3}H]DHT and increasing concentrations of radio-inert steroids. Points represent the mean of duplicate determinations of two different experiments, standard deviations are too small to be represented. DHT, P_4, CA (cyproterone acetate) and the synthesized steroids **4**—**15** are also represented in this figure.

In this study we demonstrated that radio-inert DHT, as well as increasing CA concentrations, were able to inhibit the binding of [^{3}H]DHT to the AR present in the cytosolic fraction, as shown by the K_i values of 10 and 10.8 respectively. Furthermore, some radio-inert synthetic steroids (**5**, **6**, **8**, **9**, **11**, **14**) also inhibited the binding of [^{3}H]DHT to cytosolic AR, as indicated by the respective K_i values (Table 2). In contrast, P_4 as well as the steroids **4**, **7**, **10**, **12** and **13**, did not have any inhibitory effect on cytosolic AR, as shown in Table 2 and Fig. 5.

The data on protein content in the seminal vesicles (Table 1) show that castration (control) increases the protein content, whereas T and compounds **4**, **5**, **7**, **9** and **15** tend to decrease it. These results can be explained by taking into consideration the possibility that T as well as compounds **4**, **5**, **7**, **9** and **15** may downregulate 5α-reductase synthesis and thus control the weight and functioning of the seminal vesicles. This hypothesis could be corroborated by the fact that the control, as well as compounds **6**, **8**, **10**—**14** and **3**, apparently upregulate 5α-reductase synthesis and thus increase the protein content. This however does not explain the behavior of compounds **7** and **9** (Fig. 2). This phenomenon strongly suggests that these compounds may reduce synthesis of ARs.

In the future, several functional group modifications will be carried out with the most active compounds with the purpose of improving their antiandrogenic effect and also increasing their inhibitory activity for the enzyme 5α-reductase.

Experimental

Chemicals and Radioactive Material Solvents were laboratory grade or better. Melting points were determined on a Fisher–John melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR were taken on Varian Gemini 200s and VRX-300 spectrometers, respectively. Chemical shifts are given in ppm relative to Me_4Si (δ=0) in $CDCl_3$. The abbreviations of the signal patterns are as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Mass spectra were obtained with a HP5985-B spectrometer. IR spectra were taken on a Perkin Elmer 549B and the UV spectra were recorded on a Perkin Elmer 200s spectrometer.

New England Nuclear Co. (Boston, MA, U.S.A.) provided (15, 16 ^{3}H)T ([^{3}H]T, specific activity: (85—100) Ci/mmol) and (1, 2, 6, 7-^{3}H)DHT ([^{3}H]DHT, specific activity: (110) Ci/mmol). Steraloids, Inc. (Wilton, NH, U.S.A.) supplied radio-inert T, 5α-DHT, P_4, and CA. Sigma Chemical Co. (St. Louis, MO, U.S.A.) provided $NADPH^+$.

20-Ethylenedioxy-16β-phenylpregn-5-ene-3β,17α-diol (4) Compound **15** (1 g, 24 mmol) (the preparation of this compound is described in ref. 5), dimethylsulfide–cuprous bromide complex (300 mg) and a 1 mol solution of phenylmagnesium bromide in THF (14 ml) were refluxed for 4 d under a nitrogen atmosphere. The reaction mixture was then poured into saturated aqueous ammonium chloride solution (15 ml), and the mixture extracted 3 times with chloroform. The organic phase was washed with water and dried over anhydrous sodium sulfate and the solvent removed *in vacuo*. The resulting crude product was recrystallized from methanol to give 0.71 g, 1.57 mmol (65%) of the pure compound **4** (Fig. 3). mp 220—222 °C. IR (KBr) cm^{-1}: 3400, 1600, 1450, 1048, 900, 766, 698. ^{1}H-NMR ($CDCl_3$) δ: 1.0 (3H, s), 1.10 (3H, s), 2.21 (3H, s), 3.1 (1H, m), 3.3 (2H, m), 3.52 (2H, m), 5.39 (1H, dd, J_1=4, J_2=2 Hz), 7.26 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 14.5 (C-18), 20 (C-19), 22 (C-21), 60 (C-16), 61, 64 (–OCH_2–CH_2–O–), 71 (C-17), 72 (C-3), 90 (C-20), 121 (C-6), 125—128 (C-16, Ph-), 143 (C-5). MS (*m/z*): 452 (M^+).

3β,17α-Dihydroxy-17β-methyl-16β-phenyl-D-homopregn-5-en-17a-one (5) A solution of steroid **4** (1.0 g, 2.2 mmol) in acetone (80 ml) and perchloric acid (1 ml) (Fig. 3) was stirred at room temperature for 3 h. A saturated aqueous sodium bicarbonate solution (100 ml) was added to neutralize the acid. The crude compound **5** precipitated; it was filtered and recrystallized from methanol. Yield 0.87 g, 2.1 mmol (96%), mp 209—211 °C. IR (KBr) cm^{-1}: 3482, 3426, 1692, 1600, 1050. ^{1}H-NMR ($CDCl_3$) δ: 1.0 (3H, s), 1.10 (3H, s), 1.2 (3H, s), 2.9 (1H, m), 5.39 (1H, dd, J_1=4, J_2=2 Hz), 7.26 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 16 (C-18), 19 (C-19), 24 (CH_3 at C-17), 54 (C-16), 71 (C-3), 78 (C-17), 218 (C-17a). MS (*m/z*): 409 (M^+).

In the hydrolysis of the ketal **4** an expansion of the 5-membered D-ring to a 6-membered D-homo compound took place.[16]

17α-Hydroxy-17β-methyl-16β-phenyl-D-homopregna-4,6-diene-3,17a-dione (6) To a suspension of **5** (1.0 g, 2.4 mmol), lithium carbonate (3.0 g) and lithium bromide (2.0 g) in *N,N*-dimethylformamide (14 ml) was added dropwise a solution containing bromine (0.3 ml) dissolved in dioxane (8.5 ml). The mixture was then kept a 75 °C for 2 h. The reaction mixture was allowed to cool to room temperature and the inorganic salt was filtered off. To the filtrate, a sodium bicarbonate–sodium bisulfite solution (180 ml, 0.3 g $NaHCO_3$; 0.3 g $NaHSO_3$) was added to eliminate the unreacted bromine. Upon cooling the crude product **6** (Fig. 3) precipitated. Recrystallization from acetone afforded 0.59 g, 1.46 mmol (61%) of the pure compound **6**. mp 276—280 °C. UV (nm): 282 (ε=26500). IR (KBr) cm^{-1}: 3446, 3028, 1694, 1656, 1616, 1126, 878. ^{1}H-NMR ($CDCl_3$) δ: 1.1 (3H, s), 1.2 (3H, s), 1.4 (3H, s), 3.0 (1H, d, J=3 Hz), 5.7 (1H, s), 6.2 (1H, dd, J_1=10, J_2=2 Hz), 6.3 (1H, dd, J_1=10, J_2=2 Hz), 7.26 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 116.2 (C-18), 16.4 (C-19), 24 (CH_3 at C-17), 54 (C-16), 79 (C-17), 124 (C-4), 165 (C-5), 199 (C-3), 217 (C-17a). MS (*m/z*): 404 (M^+).

17α-Acetoxy-17β-methyl-16β-phenyl-D-homopregna-4,6-diene-3,17a-dione (7) A solution containing steroid **6** (1 g, 2.4 mmol), *p*-toluenesulfonic acid (50 mg), trifluoroacetic anhydride (0.6 ml) and glacial acetic acid (1.26 ml) was stirred for 1.5 h at room temperature (nitrogen atmosphere). The reaction mixture was diluted with chloroform (10 ml) and was neutralized with an aqueous sodium bicarbonate solution to pH 7. The organic phase was separated and dried over anhydrous sodium sulfate and the solvent eliminated *in vacuo* and the crude product was purified by silica gel column chromatography. Hexane–ethyl acetate (8 : 2) eluted 750 mg, 1.68 mmol (70%) of the pure product **7**. mp 278—280 °C. UV (nm): 284 (ε=23400). IR (KBr) cm^{-1}: 1725, 1705, 1660, 1616, 1282, 875. ^{1}H-NMR ($CDCl_3$) δ: 0.8 (3H, s), 1.0 (3H, s), 1.3 (3H, s), 4.0 (1H, m), 5.7 (1H, s), 6.2 (1H, dd, J_1=10, J_2=2 Hz), 6.4 (1H, dd, J_1=10, J_2=2 Hz), 7.2 (2H, m), 7.3 (3H, m). ^{13}C-NMR ($CDCl_3$) δ: 16 (C-18), 18 (C-19), 23 (CH_3 at C-17), 50 (C-16), 84 (C-17), 122 (C-4), 160 (C-5), 165 (CH_3COO), 195 (C-3), 215 (C-17a). MS (*m/z*): 446 (M^+).

17α-Hydroxy-16β-methylpregna-1,4,6-triene-3,20-dione (8) This compound was prepared from 16-dehydropregnenolone acetate **12** (Fig. 4). The exact procedure for the preparation of intermediates **12**—**17** is described in ref. 5.

A solution of steroid **17** (1 g, 2.88 mmol) and DDQ (2.2 g) in dioxane (50 ml) was allowed to reflux for 4 d. Upon cooling, the precipitated 2,3-dichloro-5,6-dicyanohydroquinone was filtered off. To the filtrate was added 3% aqueous sodium hydroxide solution (100 ml) and chloroform (100 ml) and the mixture was stirred for 5 min. The organic phase was washed 3 times with 3% aqueous sodium hydroxide solution and water. It was then dried over anhydrous sodium sulfate and the solvent removed *in vacuo*. The crude product was purified by silica gel column chromatography. Hexane–ethyl acetate (6 : 4) eluted 640 mg, 1.88 mmol (65%) of the pure product **8**. mp 197—199 °C. UV (nm): 223, 258, 303 (ε=14800, 12800, 18100). IR (KBr) cm^{-1}: 3389, 1707, 1651, 1600. ^{1}H-NMR ($CDCl_3$) δ: 0.77 (3H, s), 1.2 (3H, d, J=2 Hz), 1.3 (3H, s), 2.3 (3H, s), 6 (1H, d), 6.2 (1H, s), 6.3 (1H, d), 6.4 (1H, J=5 Hz), 7 (1H, d, J=5 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14 (C-18), 18 (C-19), 50 (CH_3, C-16), 78 (OH, C-17), 186 (C-20), 217 (C-3). MS (*m/z*): 340 (M^+).

16β-Methyl-17α-propionyloxypregna-1,4,6-triene-3,20-dione (9) A solution containing steroid **8** (200 mg, 0.5 mmol), *p*-toluenesulfonic acid (10 mg), trifluoroacetic anhydride (1.2 ml) and propionic acid (0.9 ml) was stirred for 5 h at room temperature (nitrogen atmosphere). The reaction mixture was then diluted with chloroform (10 ml) and neutralized with an aqueous sodium bicarbonate solution to pH 7. The organic phase was separated and dried over anhydrous sodium sulfate and solvent removed *in vacuo* and the crude product was purified by silica gel column chromatography. Hexane–ethyl acetate (8 : 2) eluted 163 mg, 0.41 mmol (80%) of the pure product **9** (Fig. 4). mp 218—220 °C. UV (nm): 223, 225, 298 (ε=14800, 12600, 17800). IR (KBr) cm^{-1}: 1725, 1707, 1604. ^{1}H-NMR ($CDCl_3$) δ: 1.1 (3H, s), 1.2 (3H, S), 1.3 (3H, d, J=2 Hz), 2.2 (3H, s), 2.4 (2H, m), 6 (1H, s), 6.2 (1H, s), 6.3 (1H, J=4 Hz), 7.1 (1H, d, J=4 Hz). ^{13}C-NMR ($CDCl_3$) δ: 12 (C-18), 18 (C-19), 28 (C-21), 47 (CH_3, C-16), 83 (C-17), 173 (CH_3COO), 200 (C-20), 212 (C-3). MS (*m/z*): 398 (M^+).

17α-Acetoxy-16β-methylpregna-1,4,6-triene-3,20-dione (10) A solution containing steroid **8** (1 g, 2.5 mmol), *p*-toluenesulfonic acid (50 mg), trifluoroacetic anhydride (6 ml) and glacial acetic acid (1.2 ml) was stirred for 5 h at room temperature (nitrogen atmosphere). The reaction mixture was diluted with chloroform (10 ml) and neutralized with an aqueous sodium bicarbonate solution to pH 7. The organic phase was separated and dried over anhydrous sodium sulfate and the solvent removed *in vacuo* and the crude product was purified by silica gel column chromatography. Hexane–ethyl acetate (8 : 2) eluted 820 mg, 2.14 mmol of the pure product **10** (Fig. 4), (80%). mp 209—210 °C. UV (nm): 222, 253, 296 (ε=14900, 12700, 18000). IR (KBr) cm^{-1}: 1728, 1700, 1610. ^{1}H-NMR ($CDCl_3$) δ: 1.0 (3H, s), 1.2 (3H, S), 1.4 (3H, d, J=3 Hz), 2.2 (3H, s), 2.4 (3H, s), 6.2 (1H, s), 6.3 (1H, s), 6.4 (1H, d, J=3 Hz), 7.2 (1H, d, J=3 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14 (C-18), 20 (C-19), 27 (C-21), 48 (CH_3, C-16), 85 (C-17), 175 (CH_3COO), 190 (C-20), 210 (C-3). MS (*m/z*): 384 (M^+).

17α-Acetoxy-4-bromopregna-1,4-diene-3,20-dione (11) This compound was synthesized from 17α-acetoxyprogesterone (**18**) (Fig. 3). The preparation of the corresponding intermediates **19** and **20** is given in ref. 6.

A solution of steroid **20** (1 g, 2.2 mmol) and DDQ (3 g) in dioxane (30 ml) was stirred at room temperature. Hydrogen chloride was bubbled through the solution for 20 min and stirring was continued for 2 d at room temperature and 1 d under reflux. The reaction mixture was cooled to 0 °C and the precipitated 2,3-dichloro-5,6-dicyanohydroquinone was filtered off. To the filtrate was added 3% aqueous sodium hydroxide solution (50 ml) and chloroform (50 ml) and the mixture was stirred for 10 min. The organic phase was separated, washed 3 times with water, dried over anhydrous sodium sulfate and the solvent removed *in vacuo*. The crude product was purified by silica gel column chromatography. Hexane–ethyl acetate (8 : 2) eluted 300 mg, 0.66 mmol (30%) of the pure product **11**. mp 203—205 °C. UV (nm): 242 (ε=15200). IR (KBr) cm^{-1}: 1730, 1705, 1671, 630. ^{1}H-NMR ($CDCl_3$) δ: 0.7 (3H, s), 1.1 (3H, s), 2.0 (3H, s), 6.5 (1H, d, J=6 Hz), 6.9 (1H, d, J=6 Hz). ^{13}C-NMR ($CDCl_3$) δ: 13 (C-18), 16 (C-19), 25 (C-21), 80 (C-17), 122 (C-2), 128 (C-1), 160 (C-5), 175 (CH_3COO), 182 (C-3), 217 (C-20). MS (*m/z*): 448 (M^+).

16α,17α-Epoxy-3β-hydroxypregn-5-en-20-one (13), 3β-Acetoxy-16α,17α-epoxypregn-5-ene-20-one (14), 16α,17α-Epoxy-20-ethylenedioxypregn-5-en-3β-yl Acetate (15) These compounds were prepared from 16-dehydropregnenolone acetate (**12**) (Fig. 4). The detailed preparation of these compounds is described in ref. 5, however this publication does not report the pharmacological evaluation of these compounds.

Animals and Tissues Adult male Syrian Golden hamsters (150—200 g) were obtained from Metropolitan University-Xochimilco, Mexico. The animals were kept in a room with controlled temperature (22 °C) and light–dark periods of 12 h. Food and water were provided *ad libitum*.

Gonadectomies were performed under light ether anesthesia 15 d before treatment. Animals were sacrificed by ether anesthesia. The seminal vesicles were immediately removed, blotted, and weighed prior to use. Tissues used in the metabolic experiments were homogenized with a tissue homogenizer (model 985—370 variable speed 5000—30000 rpm, Biospec Products, Inc.).

Flank Organ Tests The flank organ test was performed as previously

reported.[17] The effect of the steroids on the flank organs of male hamsters, which were gonadectomized 15 d before the experiments, was determined on 15 groups of 4 animals/experiment, selected at random.

Daily subcutaneous injections of 200 μg of steroids **4**—**15** (Fig. 1) dissolved in 20 μl of sesame oil were administered for 3 d together with 200 μg of T. Three groups of animals were kept as controls, one was injected with 20 μl of sesame oil, the second with 200 μg of T for 3 d and the third with 200 μg of finasteride.[5,6]

After treatment, the animals were sacrificed by ether anesthesia. Both flank organs of the animal were shaven and the diameter of the pigmented spot was measured. The results were analyzed using one-way analysis of variance applying the Turkey test for comparison of the results. EPISTAT software was used for this evaluation.

Seminal Vesicles Test The effect of steroids on seminal vesicles from castrated male hamsters was determined after sacrificing the animals. The seminal vesicles were dissected out and weighed on a balance. Two separate experiments were performed for each group of steroid-treated animals. The results were analyzed using one-way analysis of variance with EPISTAT software.

***In Vitro* Metabolic Studies with Seminal Vesicles** Homogenates from male hamster seminal vesicles (4.2±0.02 mg protein) were prepared from intact adult male animals, using Krebs–Ringer-phosphate buffer solution, at pH 6. Tissue preparations were incubated[17] in duplicate with 4.14 mM [^{3}H]T in the presence of 1 μM $NADPH^+$, 0.013 μM of unlabeled T, **3**, and compounds **6**—**15** in a Dubnoff metabolic incubator at 37 °C for 60 min with O_2/CO_2 (95/5) as the gas phase. The final incubation volume was 1 ml. Incubations without tissues were used as controls. Incubations were terminated by addition of dichloromethane, and [^{3}H]steroid was extracted (4×) using 3 vol. of dichloromethane. The solvent was removed under vacuum and the resulting extract was washed with hexane to remove the remaining lipids. The protein content of the homogenates was determined by Bradford's dye-binding method[15] using bovine serum albumin (BSA) as the standard.

Isolation and purity assessment of radioactive DHT was carried out by the reverse isotope dilution technique. The isolated compound was purified with steroid carriers (T, DHT) in a thin-layer chromatographic system (chloroform–acetone, 9 : 1). The radioactive conversion product was identified on chromatographic plates by autoradiography, while non-radioactive steroid carriers were detected using phosphomolybdic acid reagent and a UV lamp (254 nm). Radioactivity was determined in a Packard 3255 liquid scintillation spectrometer, using Riafluor (Dupont, Boston, MA) as the counting solution. The counting efficiency was ^{3}H 67%. The losses of radioactivity during the procedure were calculated in agreement with the results obtained from control experiment without tissue. The conversion to DHT was calculated and expressed as pmol of DHT/g protein /h.

Antagonistic Activity of the Synthesized Steroids for ARs The antagonistic effect of steroids on the ARs of castrated male hamster seminal vesicles was determined on 100 animals (150—200 g), selected at random and gonadectomized 8 d before the experiments. The animals were kept in a room with controlled temperature (22 °C) and light–dark periods of 12 h. Food and water were provided *ad libitum*.

Cytosol Preparations for AR Competition Analysis Tissue homogenization was performed as described above using TEDAM 1 : 1 (20 mM Tris–HCl, pH 7.4 at 4 °C, 1.5 mM EDTA, 0.25 mM dithiothreitol, and 10 mM sodium molybdate) containing 10% glycerol (v/v). The homogenate was centrifuged at 105000×***g*** for 1 h at 2 °C in a SW 50.1 rotor (Beckman Instruments, Palo Alto, CA). Cytosol protein content was determined by Bradford's dye-binding method[15] using BSA as the standard.

AR competition studies were performed as described by Cabeza *et al.*[19] and are briefly summarized below.

Competition Studies For competition experiments, tubes contained 1 nM[20] [^{3}H]DHT (specific activity 110 Ci/mmol) plus a range of increasing concentrations (20—200 nM) of 5-α DHT, CA, and steroids **5**—**15**. Aliquots (150 μl) of cytosol were added and incubated (in duplicate) for 18—20 h at 2—4 °C in the tubes described above. In another experiment, parallel sets of tubes containing identical concentrations of [^{3}H]DHT plus a 100-fold excess of radio inert steroids were used dextran-coated charcoal in TEDAM buffer 800 μl was added and the mixture was incubated for 10 min at 4 °C. The dextran was agitated during 30 min before addition of the charcoal to the mixture. The tubes were vortexed and immediately centrifuged at 800×***g*** for 10 min; aliquots (200 μl) were subsequently submitted and the radioactivity was evaluated. Specific binding was determined by subtracting the mean disintegration per minute (dpm) in the presence of excess unlabeled steroids from the mean dpm of corresponding tubes containing only [^{3}H]DHT.

Results were analyzed using one-way analysis of variance with EPISTAT software. The inhibition constant (K_i) of each compound was calculated according to the procedures described by Cheng and Prusoff.[21]

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