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Señores

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES

E. _____ S. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Solicitud de patente de invención titulada: "17-METILEN ESTEROIDES 4-HALOGENADOS, MÉTODO PARA SU PREPARACIÓN Y COMPOSICIONES FARMACÉUTICAS QUE CONTIENEN ESTOS COMPUESTOS."

Solicitante: SCHERING AKTIENGESELLSCHAFT.

N. Ref.: P2003/185

RESPUESTA AL OFICIO No. 5977 NOTIFICADO POR FIJACIÓN EN LISTA No. 145,
DE FECHA 9 DE MAYO DE 2008
(SUMINISTRO DE INFORMACIÓN)

J. IAN RAISBECK, mayor de edad y vecino de Bogotá, identificado tal como aparece al pie de mi firma, en mi carácter de en mi carácter de apoderado de la sociedad solicitante, me permito manifestar lo siguiente ante el auto emanado por ese Despacho, dentro del plazo legal previsto en el artículo 46 de la Decisión 486:

1. Presento copia de los siguientes documentos solicitados por el Examinador, según los resultados del examen de búsqueda de anterioridades, de acuerdo a lo previsto en el Artículo 46 de la Decisión 486 de la Comisión de la Comunidad Andina:

D1: CHEMICAL ABSTRACTS, vol. 62, no. 10, 10, May, 1965 (1965-05-10) Columbus, Ohio, US; abstract no. 11871e, K. BARNIKOL-OETTLER ET AL: "Wittig reaction of steroid ketones" XP002187567 & J. PRAKT CHEM., Bd. 27, Nr. 1-2, 1965, Seiten 18-33.

2

D2: CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; JARMAN, MICHAEL ET AL: "Evaluation of some 4-fluoro- and 4-cyano derivatives of delta 4, 3-ketosteroids as inhibitors of testosterone 5-alpha- reductase" retrieved from STN Database accession no. 122:1349 XP002187568 & J. ENZYME INHIB. (1994), 8(1), 17-23.

D3: XUN LI: "Synthesis and in vitro evaluation of 4-substituted N-(1,1 dimethylethynil)-3-oxo-4-androstenr-17 beta carboxamida as 5 - alfa reductase inhibitors and antiandrogens" J Med Chem 1995, 38(9),1456-61.

D4: CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; GUARNA, A. ET AL: "Relationship between structure and activity of 5-alpha- reductase inhibitors" retrieved from STN Database accession no. 123:102007 XP002187570 & INT. CONGR. SER. (1994), 1064 (SEX HORMONES AND ANTIHORMONES IN ENDOCRINE DEPENDENT PATHOLOGY), 93-108.

Atentamente,



J. IAN RAISBECK

C.C. 79'376.996 de Bogotá D.C.

T.P . 135.799 del C. S. de la J.

Anexo: Artículos mencionados.

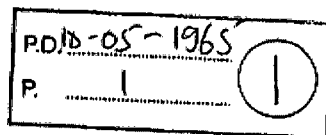
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6001 Chemical Abstracts, Columbus, Ohio, US

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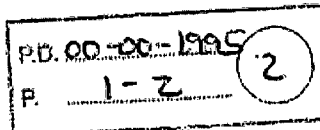
239



Wittig reaction of steroid ketones. Kurt Barnikol-Oettler, Rudolf Zepter, and Karl Heller. *J. Prakt. Chem.* 27(1-2), 18-33 (1965)(Ger). The reactions of several 6- and 17-oxo steroids with $[\text{Ph}_3\text{PMe}] \text{Br}$ and PhLi or NaNH_2 are described; CO groups not to be olefinated were protected by ketalization. The 6-oxo steroids of the 5 α -series were converted in this manner in good yields into the 6-methylene analogs. The olefination of the 6-position in the 5 β -steroids, however, was more difficult because of steric effects. 5 β -Androstane-3,6 α ,17 β -triol 17-acetate (5 g.) in 50 cc. 80% AcOH treated at 8-10° for 18 min. with 3.8 g. CrO_3 in 40 cc. 80% AcOH and kept 3.5 hrs. at 15° yielded 2.39 g. 5 β -androstane-17 β -ol-3,6-dione 17-acetate (I), m. 150-4° and 161.5-63° (Me_2CO), $[\alpha]_D -114^\circ$ (all rotations were measured in CHCl_3). I (1.3 g.) in 13 cc. 2-ethyl-2-methyl-1,3-dioxolane (II) treated at 100° with 50.7 mg. $p\text{-MeC}_6\text{H}_4\text{SO}_3\text{H}$ and cooled after 5 min. to below 0° yielded 1.18 g. 3-ethylene ketal (III) of I, m. 166-6.8° (MeOH), $[\alpha]_D -55.7^\circ$. III (900 mg.), 900 mg. K_2CO_3 , 18 cc. MeOH , and 3.7 cc. H_2O refluxed 1.5 hrs. gave 629 mg. 3-ethylene ketal (IV) of 5 α -androstane-17 β -ol-3,6-dione (V), m. 188.2-8.7° (MeOH), $[\alpha]_D -22^\circ$. I (393 mg.) in 13 cc. MeOH refluxed 2 hrs. with 400 mg. K_2CO_3 and 1.2 cc. H_2O gave 247 mg. V, m. 234-6° (Me_2CO), $[\alpha]_D -8^\circ$ (CHCl_3). V (800 mg.) in 17 cc. II heated 4 min. at 100° with 36 mg. $p\text{-MeC}_6\text{H}_4\text{SO}_3\text{H}$ gave 516 mg. IV, m. 188-8.7° (MeOH), $[\alpha]_D -22^\circ$, and about 80 mg. mixt. of V, IV, and the diketal. $[\text{Ph}_3\text{PMe}] \text{Br}$ (13 g.) and 144 cc. dry Et_2O treated with stirring at room temp. with 72 cc. Et_2O contg. 3 g. PhLi and then with 2.5 g. IV in 14 cc. dry tetrahydrofuran (THF) and 72 cc. dry Et_2O stirred 4 hrs. at room temp., and kept overnight, 200 cc. Et_2O replaced by 220 cc. THF, and refluxed 1 hr., and the product chromatographed on 150 g. silica gel yielded 1.92 g. 3-ethylene ketal (VI) of 6-methylene-5 α -androstane-17 β -ol-3-one (VII), m. 181.5-82° (MeOH), $[\alpha]_D -16.6^\circ$. VI (1 g.) in 70 cc. Me_2CO refluxed 2 hrs. with 8.5 cc. 2N H_2SO_4 gave 723 mg. VII, m. 196-7.2° (Me_2CO), $[\alpha]_D -25.5^\circ$; acetate (VIII) m. 187.5-8.5°, $[\alpha]_D -27.5^\circ$. VIII (523 mg.) in 5.85 cc. $\text{C}_6\text{H}_5\text{N}$ stirred 4 hrs. at room temp. with 406 mg. OsO_4 , treated with 720 mg. NaHSO_3 in 11.6 cc. H_2O and 7.8 cc. $\text{C}_6\text{H}_5\text{N}$, and stirred 0.5 hr. gave 445.6 mg. 6 β -hydroxymethyl-5 α -androstane-6 α ,17 β -diol-3-one (IX), m. 174-8.2° (Me_2CO), $[\alpha]_D -1^\circ$. IX with $\text{Ac}_2\text{O}-\text{C}_6\text{H}_5\text{N}$ gave 6 β -acetoxymethyl-17 β -acetoxymethyl-5 α -androstane-6 α -ol-3-one, m. 142.5-3.5°, $[\alpha]_D 6^\circ$. Diacetate (30 g.) of 24,24-diphenyl-5 β -chol-23-ene-3,6 α -diol (X) in 600 cc. MeOH refluxed 2 hrs. with 30 g. K_2CO_3 in 90 cc. H_2O gave 32.5 g. X, m. 180-6°. X (25.5 g.) in 400 cc. $\text{C}_6\text{H}_5\text{N}$ treated during 2 hrs. with stirring at room temp. with 10.45 g. CrO_3 , stirred 1 hr., and kept overnight yielded 14.5 g. mixt., m. 108-13° (MeOH); an 8-g. portion chromatographed on 200 g. silica gel gave 1.8 g. 24,24-diphenyl-5 β -chol-23-ene-3,6-dione, m. 148-64°, $[\alpha]_D -33.8^\circ$, and 3.2 g. 24,24-diphenyl-5 β -chol-23-en-3 α -ol-6-one (XI), m. 145-8°, $[\alpha]_D -8^\circ$. XI (983 mg.) in 30 cc. MeOH refluxed 2 hrs. with 1 g. K_2CO_3 in 3 cc. H_2O gave 614 mg. Δ^5 -epimer of XI, m. 201-5° ($\text{MeOH}-\text{Me}_2\text{CO}$), $[\alpha]_D 27.5^\circ$. $[\text{Ph}_3\text{PMe}] \text{Br}$ (13.35 g.) added during 1 hr. under argon to NaNH_2 prep'd. from 940 mg. Na and 170 cc. liquid NH_3 , the NH_3 replaced by 60 cc. dry Et_2O , and the mixt. dild. with an addnl. 60 cc. Et_2O , treated with 2.5 g. 4-chloro-4-androstene-3,17-dione 3-ethylene ketal in 90 cc. dry THF, stirred 2 hrs. at room temp., kept overnight, concd. during 15 min. to remove 150 cc. solvent, dild. with 60 cc. dry THF, and refluxed 15 min. gave 0.37 g. unreacted ketal, m. 230-4°, and 1.65 g. 17-methylene-4-chloro-4-androstene-3-one 3-ethylene ketal (XII), m. 181.5-83° (Me_2CO), $[\alpha]_D 133^\circ$. XII (1 g.) in 60 cc. Me_2CO refluxed 2 hrs. with 8.5 cc. 2N H_2SO_4 gave 710 mg. 4-chloro-17-methylene-4-androstene-3-one, m. 138.4-40° (MeOH), $[\alpha]_D 150^\circ$. 17-Methylene-5-androstene-3-one 3-ethylene ketal (1.48 g.) in 90 cc. Me_2CO refluxed 2 hrs. with 2N H_2SO_4 gave 84% 17-methylene-4-androstene-3-one, m. 133.6-35°, $[\alpha]_D 125^\circ$.

F. W. Hoffmann

XP-002187568



8/25 - (C) FILE HCAPLUS

STN CA Caesar accession number : 1182

AN - 1995:236746 HCAPLUS

DN - 122:1349

TI - Evaluation of some 4-fluoro- and 4-cyano derivatives of .DELTA.4, 3-ketosteroids as inhibitors of testosterone 5.alpha.- reductase

IN - Jarman, Michael; Barrie, S. Elaine; Houghton, John; Rowlands, Martin G.; Mann, John; Haase-Held, Margret; Hatzis, Maria

CS - CRC Laboratory, Institute Cancer Research, Sutton, Surrey, SM2 5NG, UK

SO - J. Enzyme Inhib. (1994), 8(1), 17-23

CODEN: ENINEG; ISSN: 8755-5093

DT - Journal

LA - English

AB - Some 4-fluorinated analogs of 3-oxo-.DELTA.4 steroids and 4-cyano derivs. of progesterone and androstenedione were evaluated as inhibitors of steroid, 5.alpha.- reductase activity. Inhibitors of this enzyme may be useful in treating prostatic cancer. 4-Fluoroandrostenedione was a modest inhibitor of the rat enzyme (IC50 = 4.08 .mu.M), while 4-cyanoprogestosterone was a potent inhibitor of both the rat and human enzymes (IC50 values = 0.045 .mu.M and 0.050 .mu.M resp.). These two steroids were tested in vivo for activity against androgen sensitive organs in WHT mice. 4-Fluoroandrostenedione caused increases in organ wts., suggesting it is an androgen agonist, while the 4-cyano compd. displayed modest androgen ablation. Therefore substitutions at the 4-position may produce compds. of therapeutic use in treating prostate cancer.

IT - 1649-25-8 , 4-Fluorotestosterone 1881-31-8

1995-24-0 98102-30-8

RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses) (ketosteroid fluoro and cyano derivs. as testosterone reductase inhibitor and as neoplasm inhibitors in prostate cancer)

RN - 1649-25-8 HCAPLUS

CN - Androst-4-en-3-one, 4-fluoro-17-hydroxy-, (17.beta.)- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

[--00000048]

RN 1881-31-8 HCAPLUS

CN Estr-4-en-3-one, 4-fluoro-17-hydroxy-, (17.beta.)- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

[--00000049]

RN 1995-24-0 HCAPLUS

CN Pregn-4-ene-3,20-dione, 4-fluoro- (7CI, 8CI, 9CI) (CA INDEX NAME)

Absolute stereochemistry.

[--00000050]

RN 98102-30-8 HCAPLUS

CN Androst-4-ene-3,17-dione, 4-fluoro- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

[--00000051]

IT ***9081-34-9*** , Testosterone 5.alpha.- ***reductase***

RL: BSU (Biological study, unclassified); BIOL (Biological study) (ketosteroid fluoro and cyano derivs. as testosterone

reductase inhibitor and as neoplasm inhibitors in
prostate cancer)
RN 9081-34-9 HCAPLUS
CN Reductase, steroid 5.alpha.- (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

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241

Synthesis and *in Vitro* Evaluation of 4-Substituted *N*-(1,1-Dimethylethyl)-3-oxo-4-androstene-17 β -carboxamides as 5 α -Reductase Inhibitors and Antiandrogens

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Medicinal Chemistry Division, Laboratory of Molecular Endocrinology, CHUL Research Center, Québec G1V 4G2, Canada

Received July 29, 1994*

4-Substituted *N*-(1,1-dimethylethyl)-3-oxo-4-androstene-17 β -carboxamides with the hydroxy (OH) 3d, mercapto (SH) 3e, chloro (Cl) 3f, and bromo (Br) 3g substituents at the 4-position were prepared in a two-step sequence with overall yields of 21%, 27%, 41%, and 37%, respectively. Compounds 3d-g showed weak inhibitory activity on human type I 5 α -reductase ($IC_{50} \geq 700$ nM) while they had intermediate inhibitory activity on human type II 5 α -reductase (IC_{50} s of 172, 437, 192, and 387 nM, respectively). In androgen-sensitive Shionogi cells, the inhibition of dihydrotestosterone (DHT) stimulatory action on the proliferation of the androgen-sensitive cancer cells by all four compounds was high at IC_{50} s of 170-279 nM compared with 117 nM for hydroxyflutamide. The present data show compounds having both moderate inhibition of human type II 5 α -reductase activity and relatively potent antiandrogenic action, two beneficial characteristics in the therapy of androgenic-sensitive diseases.

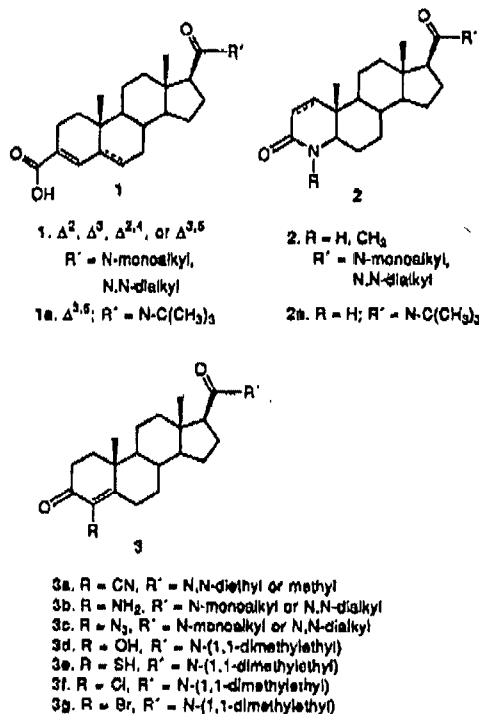
Introduction

Study of the mechanisms and inhibition of steroid 5 α -reductase (Δ^4 -3-oxosteroid 5 α -oxidoreductase; EC 1.3.99.5)¹ is a topic of major current biological and pharmaceutical interest, since this enzyme catalyzes the irreversible reduction of the C-4-C-5 double bond of 3-oxo-4-ene steroids to their corresponding 5 α -3-oxosteroid derivatives,² using a nicotinamide adenine dinucleotide hydrogen phosphate (NADPH) as co-factor.³ The main interest in 5 α -reductase arises from its role in the conversion of testosterone (T) to dihydrotestosterone (DHT), the most active androgen known to be essential for male differentiation,⁴ growth and function of androgen-sensitive sex organs and in the pathophysiology of many diseases, especially benign prostatic hyperplasia (BPH), prostate cancer,⁵ acne,⁶ hirsutism,⁷ and androgenic alopecia (male-pattern baldness).⁸

Recently, the isolation and characterization of human types I and II 5 α -reductase genes have been described.⁹ The human type II 5 α -reductase is presumed to be involved in the prostatic androgen-related diseases mentioned above. However, human type I 5 α -reductase encodes for the isozyme presumed to be expressed in the skin. A rationale treatment of these androgen-sensitive conditions would be the selective inhibition of either one or both enzymes with specific inhibitors.

Although several steroidal and nonsteroidal compounds¹⁰ have been reported as inhibitors of 5 α -reductase during the last decade, steroidal compounds have attracted more attention. It has been observed that for a compound to be a good to excellent inhibitor, a conjugated system ($sp^2-sp^2-sp^2$) should be present at the C-3, -4, and -5 positions of the A ring of steroids together with a lipophilic group at the C-17 position.^{11,12} On the basis of these observations, remarkable progress has been made in the design and synthesis of steroidal

Chart 1



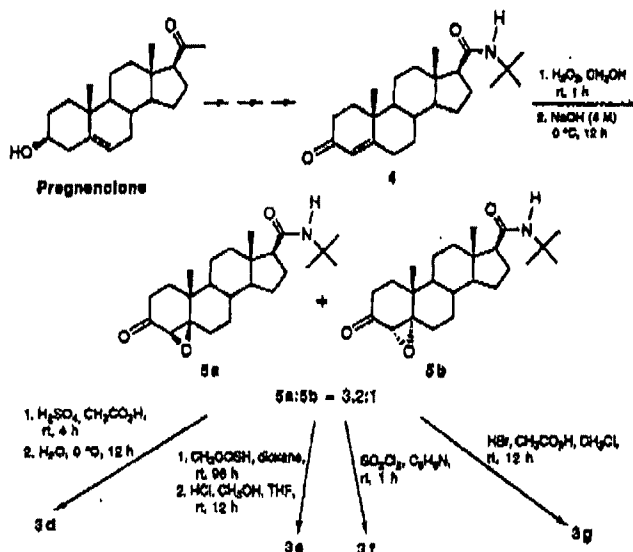
inhibitors, by selectively modifying the 3-, 4-, 5-, and 17-positions of steroids: the two best known series of compounds are 3-carboxy steroids 1,¹¹ and 4-aza steroids 2 (Chart 1).¹² The *in vitro* evaluation of compounds 1 on inhibitory activity of human and rat type II prostatic steroid 5 α -reductases has shown that 17 β -carbamoyl-3-androstene-3-carboxylic acids are highly potent inhibitors of the enzyme. The inhibitory activity is enhanced in analogues possessing an additional C-5-C-6 double bond while it is decreased in analogues

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* Abstract published in *Advance ACS Abstracts*, April 1, 1995.

X 243

Scheme 1



lacking the C-3–C-4 double bond.^{11d} Epristeride (1a) of this series, which is under clinical trials, reduces the serum DHT levels by 25–54% at 0.4–160 mg doses after 8 days.¹³ It is proposed that these unsaturated steroidal acid functions mimic the putative enolate intermediate of the enzymatic process.^{11a,b} However, 3-carboxyaryl isomers of 1 showed significantly reduced activity with the rodent enzyme relative to the human 5 α -reductase.^{11c}

Another series of mechanism-based steroidal inhibitors are the 4-azasteroids, which were prepared by structural modifications of the A ring and of moieties attached at the C-17 position of the steroid nucleus. High *in vitro* activity was seen with 4-methyl-4-aza derivatives on both human and rat type II prostatic 5 α -reductases. However, 4-unsubstituted 4-aza derivatives are usually less active than 4-methyl derivatives.^{12a} The activity-enhancing features of a 4-methyl group and a semipolar side chain at the 17-position indicate that the enzyme has steric and lipophilic preferences at these positions.^{12c} One of the well-known compounds in this series is Finasteride (2a) (PROSCAR) which has the above-indicated substituents in the A and D rings. Potent *in vitro* inhibition was verified for both human and rat type II enzymes. Clinical trials in patients with BPH, using Finasteride, have demonstrated its therapeutic efficacy in BPH with an 80% reduction in circulating DHT levels and a 28% reduction in prostate size.^{12b–d} It is believed that these compounds behave as "intermediate state" inhibitors and the A-ring lactam of 3-oxo-4-azasteroids mimics the conformation of the intermediate enol form.¹⁴ Recently, another series of azasteroids were prepared and evaluated for their inhibitory activities. The 6-azasteroids were found to be active on both types I and II 5 α -reductases.¹⁵

4-Substituted 3-oxo-4-androstenes having an *N*-(1,1-dimethylethyl)-17 β -carboxamide group were prepared and tested *in vitro* as human type II 5 α -reductase inhibitors.¹⁶ The 4-cyano analogue 3a showed high *in vitro* potency (IC_{50} = 2.9 nM). However, when a 4-amino or 4-azido group was present, a decrease in

activity was observed (IC_{50} = 54 nM for 3b).^{14b,c} It thus appears that the steroids having a 4-substituted 3-oxo group in the A ring and a 17 β -carboxamide substituent in the D ring have good to excellent 5 α -reductase inhibitory activity. The 4-fluoro and 4-cyano derivatives of androstenedione and progesterone were also evaluated on the type II enzyme.¹⁷ The fluoro compounds exhibited weak *in vitro* activity and were androgen agonists *in vivo*, while the cyano compounds were potent inhibitors.

To better understand structure–activity relationships in connection with both types of human 5 α -reductases, we have synthesized and assayed the *in vitro* activity of 4-substituted (OH, SH, Cl and Br) 3-oxo-4-androstene-17 β -carboxamides as inhibitors of both types of human 5 α -reductases (types I and II). These compounds were also evaluated for their inhibitory activities of the androgen-induced proliferation of Shionogi cells.

Results and Discussion

Chemistry. The 4-substituted 3-oxo-4-ene-steroid-17 β -carboxamides were prepared from the key intermediate, 3-oxo-4-ene-steroid-17 β -carboxamide 4 while compound 4 was prepared from commercially available pregnenolone (Scheme 1).^{12a} Thus, *N*-(1,1-dimethylethyl)-3-oxo-4-androstene-17 β -carboxamide (4) was obtained in 89% yield from a one-pot reaction, in which the 17 β -carboxylic acid was first converted to 3-oxo-4-androstene-17 β -carboxylic chloride *in situ* by stirring with oxalyl chloride in pyridine at –10 °C and then with *tert*-butylamine in toluene.^{12a,18} Treatment of the amide 4 with 30% hydrogen peroxide in methanol at room temperature for 1 h followed by 4 M sodium hydroxide at 0 °C for 12 h provided an isomeric mixture of 4,5-epoxides 5a/5b ($\beta\alpha$ = 3.2:1).^{19a} The isomeric ratio was determined by ¹H NMR spectroscopy. Reaction of epoxides with a mixture of concentrated sulfuric acid¹⁹ and acetic acid^{19a} gave *in situ* 4-oxyacetate which, after subsequent hydrolysis, yielded 4-hydroxy-3-oxo-4-androstene-17 β -carboxamide (3d) at a 40% yield.^{19a,d} The

Table 1. *In Vitro* Activity of 4-Substituted *N*-(1,1-Dimethylethyl)-3-oxo-4-androstene-17 β -carboxamides

entry	R	<i>in vitro</i> bioactivity ^a (IC ₅₀ , nM)		
		human type I 5 α -R (transfected 293 cells)	human type II 5 α -R (transfected SW-13 cells)	DHT ^d -stimulated Shionogi cells
finasteride (2a) ^b		218	8.47	NA
Flu-OH ^c		NA	NA	117
Δ^4 -dione ^d		2700	83.9	NA
3d	OH	>1000	172	242
3e	SH	709	437	279
3f	Cl	>1000	192	170
3g	Br	981	387	184

^a The results of the inhibition of 5 α -reductase (types I and II) and DHT-stimulated proliferation of Shionogi cells were obtained by following standard procedures described in the Experimental Section. The concentration of the compounds required to inhibit 5 α -reductase activity or 5 α -dihydrotestosterone-induced stimulation of Shionogi cells proliferation by 50% is represented as IC₅₀ values. ^b Finasteride^{12a,d} was used as a standard reference for the inhibition of 5 α -reductase. ^c Hydroxyflutamide (Flu-OH) was added as a standard control for the antiandrogen test. ^d 5 α -Dihydrotestosterone (DHT, 0.3 nM) was used as a standard substrate for the growth assay. ^e [³H]- Δ^4 -Androstenedione ([³H]- Δ^4 -dione, 2.5 nM) was added as the substrate for 5 α -reductases.

6 α -proton of compound 3d resonated at 3.0 ppm, which is a characteristic signal for the 4-substituted 3-oxo-4-ene system. In a similar fashion, 4-mercapto-3-oxo-4-androstene-17 β -carboxamide (3e) was also obtained in 50% yield, after stirring 4,5-epoxides 5a/5b with thioacetic acid.^{19a-c} Both ¹H and ¹³C NMR spectra were in agreement with the assigned structure. When the epoxides 5a/5b were treated with a mild chlorinating reagent, such as sulfuryl chloride (SO₂Cl₂), in dry pyridine at room temperature, 4-chloro-3-oxo-4-androstene-17 β -carboxamide (3f) was formed in 77% yield.^{20a,c} However, the preparation of compound 3f from 3d or 3e^{20b} under acidic conditions gave more side products. 4-Bromo-3-oxo-4-androstene-17 β -carboxamide (3g) was prepared in 69% yield from 5a/5b and hydrogen bromide in a mixture of acetic acid and chloroform at room temperature.^{20b}

Inhibition of Human 5 α -Reductase (Types I and II). Finasteride (2a) was used as the standard reference in both *in vitro* protocols. In comparison with finasteride (IC₅₀ = 218 nM), 4-substituted 3-oxo-4-androstene-17 β -carboxamides showed little or no inhibitory effect on human type I 5 α -reductase. In fact, the IC₅₀ value of each compound was at approximately or above 700 nM (Table 1). However, intermediate inhibitory activity was seen on human type II 5 α -reductase (Figure 1). Thus, the halogenated compounds 3d (IC₅₀ = 172 nM) and 3f (IC₅₀ = 192 nM) were 2-fold more potent than the thiol 3e (IC₅₀ = 437 nM) and the hydroxy compound 3g (IC₅₀ = 387 nM). However, all compounds were 20 times or above less potent than finasteride (IC₅₀ = 8.47 nM).

Inhibition of the Proliferation of Androgen-Sensitive Shionogi Canceroma Cells (Clone SEM-107). The antiandrogenic activity was determined by inhibiting DHT action on the proliferation of the androgen-sensitive Shionogi cells. Hydroxyflutamide was used as the standard reference with an IC₅₀ value of 117 nM. The inhibitory ability of the 4-substituted 3-oxo-4-androstene-17 β -carboxamides was dependent on the nature of the substituent at 4-position of the A ring (Table 1). [The 4-halogenated compounds 3f and 3g have

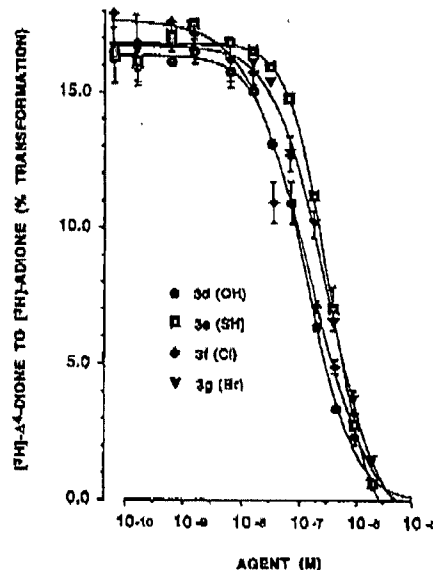


Figure 1. Inhibition of human type II 5 α -reductase by 4-substituted 3-oxo-4-androstene-17 β -carboxamides.

high inhibitory activity (IC₅₀s = 170 and 184 nM, respectively) on the proliferation of DHT-stimulated Shionogi cells. However, the 4-hydroxy compound (3d) and the thiol (3e) have some what lower potency (IC₅₀s = 242 and 297 nM, respectively) (Figure 2). This study reveals that 4-substituted compounds are androgen antagonists and are less active compared to hydroxyflutamide.

Conclusion

Compounds 3d-g are intermediate inhibitors of human type II 5 α -reductase. However, they are very weak inhibitors of human type I 5 α -reductase. In addition, these compounds show relatively good inhibitory activity on the proliferation of androgen-sensitive cells, thus

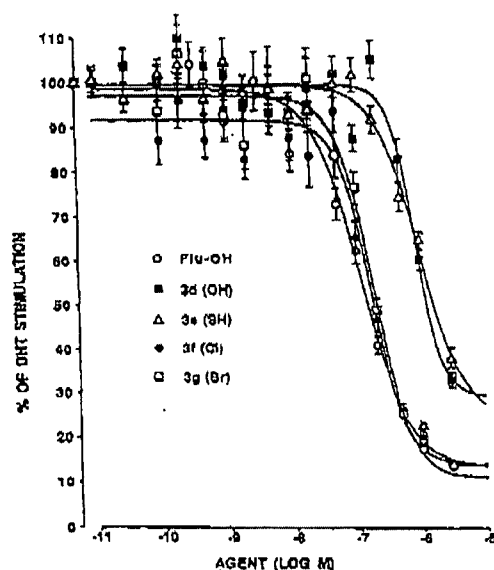


Figure 2. Inhibition of DHT-stimulated proliferation of androgen-sensitive Shionogi cells (clone SEM 107) by 4-substituted 3-oxo-4-androstene-17 β -carboxamides.

adding to the positive characteristics of these compounds. These observations should lead to design of more potent and selective inhibitors of androgenic formation and action.

Experimental Section

General. Unless otherwise indicated, materials obtained from commercial suppliers were used without further purification. Diethyl ether and tetrahydrofuran (THF) were distilled from sodium/benzophenone immediately prior to use. All reactions except those involving water as a reagent were conducted under argon atmosphere. Melting points were measured on a Gallenkamp capillary melting point apparatus and are uncorrected. IR spectra were determined with a Perkin-Elmer 1600 Series FT infrared spectrometer. ^1H NMR spectra were determined on a Bruker Aspect-3000 (300 MHz). ^{13}C NMR spectra were measured at 76.14 MHz with a Bruker Aspect-3000. Low-resolution mass spectra were obtained with a Varian Model 9700 gas chromatography/micromass 16F mass spectrometer. High-resolution mass spectra were measured at the Department of Chemistry, University of Montreal, Montreal, Quebec. Combustion analyses (C, H, N, S, Cl, Br) were performed by Galbraith Laboratories (Knoxville, TN). All the final products were at least 99.5% pure, and the purity was determined by a high-performance liquid chromatography (HPLC) of Waters Model 600E (Millipore).

N-(1,1-Dimethylethyl)-3-oxo-4-androstene-17 β -carboxamide (4) was prepared by the method of Rasmussen et al.^{12c,18}

N-(1,1-Dimethylethyl)-3-oxo-4,5-epoxyandrostane-17 β -carboxamides (5a/5b). In a 1-L flask equipped with a magnetic stir bar, a septum and an argon inlet was placed *N*-tert-butyl-3-oxo-4-androstene-17 β -carboxamide (14.0 g, 37.7 mmol) in a 1:10 mixture of methylene chloride/methanol. Hydrogen peroxide (30% aqueous solution, 19.8 mL, 174.3 mmol) was added dropwise over a period of 30 min to the above mixture. After 80 min of stirring at room temperature, the flask was cooled to 0 °C and 4 M aqueous sodium hydroxide (6.8 mL) was added dropwise. The reaction mixture was left for 12 h at 0–4 °C; after that, it was neutralized with 1 N aqueous HCl. The reaction mixture was concentrated on a rotary evaporator to a volume of about 60 mL. Ice-water (500 mL) was added, and the mixture was stirred for 15 min to

give precipitate which was filtered, washed with water, and dried under vacuum to afford the product (8.2 g, 53% yield). The analysis of the NMR spectrum gave a 3.2:1 mixture of β : α -isomer: mp 84–96 °C; IR (KBr, cm^{-1}) 3447 (sh), 3398, 2942, 2847, 1711, 1678, 1513, 1451, 1390, 1383, 1261, 1218, 862; ^1H NMR (CDCl_3) δ 0.67 (s, 2.28 H, 18-CH $_3$), 1.04 (s, 0.72 H, 18-CH $_3$), 1.06 (s, 0.72 H, 19-CH $_3$), 1.13 (s, 2.28 H, 19-CH $_3$), 0.8–1.17 (m, 2H), 1.18–1.27 (m, 3H), 1.31 (s, 9 H, 3 CH $_3$), 1.40–1.58 (m, 4 H), 1.67 (dd, J = 8.2, 13.0 Hz, 2H), 1.74–2.09 (m, 4 H), 2.11–2.42 (m, 5 H), 2.95 (s, 0.76 H, 4 α -H), 3.01 (s, 0.24 H, 4 β -H), 6.10 (br s, 1 H, NH); ^{13}C NMR (CDCl_3) (β)/ α) δ 206.8, 171.6, 70.3/70.1, 62.8/62.7, 57.6/57.5, 55.6/55.4, 51.1/50.8, 46.5, 43.7, 38.5/38.3, 37.2/37.1, 35.4/35.0, 33.1/32.5, 30.3/29.7, 29.7/29.6, 29.0 (3C), 26.2, 24.4, 23.2, 21.5/21.4, 18.9, 16.5, 13.1; EI-MS m/z (relative intensity) 387 (M^+ , 100), 372 (10), 332 (16), 215 (16), 141 (25), 128 (86), 115 (44), 95 (29), 72 (57); HRMS calcd for $\text{C}_{24}\text{H}_{36}\text{O}_3\text{N}$, 387.2773, found 387.2769. Anal. ($\text{C}_{24}\text{H}_{36}\text{O}_3\text{N}$) C, H, N.

N-(1,1-Dimethylethyl)-4-hydroxy-3-oxo-4-androstene-17 β -carboxamide (3d). Epoxides 5a/5b (1.0 g, 2.59 mmol) was added in small portions to a well-stirred mixture of concentrated sulfuric acid (0.16 mL) and glacial acetic acid (7.9 mL). The mixture, which turned progressively orange, was then stirred for 4 h at room temperature under argon. The mixture was poured into a beaker containing 250 g of ice and allowed to stand at 4 °C for 12 h. The aqueous solution was extracted with methylene chloride (4 $\times$ 120 mL). The combined organic phase was washed with brine (100 mL), dried, and concentrated to oil. The crude product was purified by silica gel flash column chromatography ($\text{C}_6\text{H}_5\text{EtOAc}:\text{CH}_3\text{COCH}_3$, 80:15:5) to give the product 3d (0.397 g, 40% yield): mp 163–165 °C; IR (KBr, cm^{-1}) 3408, 2981, 2842, 1684, 1635 (sh), 1509, 1481, 1378, 1248, 1202; ^1H NMR (CDCl_3) δ 0.70 (s, 3 H, 18-CH $_3$), 0.98–1.12 (m, 4 H), 1.16 (s, 3 H, 19-CH $_3$), 1.25 (ddd, J = 4.7, 11.9, 12.0 Hz, 1 H), 1.32 (s, 9 H, 3 CH $_3$), 1.48 (ddd, J = 3.4, 19.3, 13.1 Hz, 1 H), 1.58 (ddd, J = 3.5, 8.9, 10.5 Hz, 1 H), 1.61–1.76 (m, 4 H), 1.82–2.02 (m, 5 H), 2.10–2.20 (m, 1H), 2.22–2.52 (m, 2 H), 3.0 (dq, J = 2.2, 4.3, 14.9 Hz, 1 H, 6 α -H), 5.09 (br s, 1 H, NH), 6.08 (s, 1 H, 4-OH); ^{13}C NMR (CDCl_3) δ 193.5, 171.7, 141.2, 139.8, 57.5, 56.7, 54.2, 51.0, 48.5, 38.5, 37.8, 35.2, 34.7, 31.8, 30.9, 29.0 (3 C), 24.4, 23.3, 22.9, 20.9, 17.1, 13.1; EI-MS m/z (relative intensity) 387 (M^+ , 100), 372 (7), 314 (5), 286 (8), 250 (10), 230 (6), 147 (44), 128 (14), 93 (15), 72 (19); HRMS calcd for $\text{C}_{24}\text{H}_{36}\text{O}_3\text{N}$, 387.2773, found 387.2744. Anal. ($\text{C}_{24}\text{H}_{36}\text{O}_3\text{N}$) C, H, N.

N-(1,1-Dimethylethyl)-4-methoxy-3-oxo-4-androstene-17 β -carboxamide (3e). Thioacetic acid (0.25 mL) in dioxane (0.8 mL) was added to an ice-cold solution of epoxides 5a/5b (1.0 g, 2.59 mmol) in dioxane (8.3 mL). After 4 days of stirring at room temperature under argon, another batch of thioacetic acid (0.25 mL) was added to the dark mixture and stirred for 24 h at room temperature. The mixture was concentrated on a rotary evaporator (equipped with a vacuum pump) to give a crude 4-acetylthio compound, which was dissolved in a 1:1 mixture of methanol/tetrahydrofuran (50 mL). Concentrated HCl (0.34 mL) was added, and the mixture was stirred for 12 h at room temperature. Concentrated HCl (0.34 mL) was again added, and the mixture was stirred for 24 h at room temperature. The mixture was then poured into crushed ice-water (80 mL). The aq solution was extracted with methylene chloride (4 $\times$ 120 mL). The combined organic phase was washed with brine (100 mL), dried and concentrated to oil. The crude product was purified by silica gel flash column chromatography ($\text{C}_6\text{H}_5\text{EtOAc}:\text{CH}_3\text{COCH}_3$, 80:15:5) to give the product 3e (0.521 g, 50% yield): mp 152–154 °C; IR (KBr, cm^{-1}) 3389, 2985, 2854, 1676, 1653 (sh), 1572, 1514, 1452, 1387, 1367, 1294, 1253, 1221, 1098, 938, 892; ^1H NMR (CDCl_3) δ 0.72 (s, 3H, 18-CH $_3$), 0.93–1.10 (m, 4 H), 1.18 (s, 3 H, 19-CH $_3$), 1.27 (ddd, J = 4.5, 11.6, 11.6 Hz, 1 H), 1.34 (s, 9 H, 3 CH $_3$), 1.43 (dd, J = 2.8, 3.6 Hz, 1 H), 1.51–1.71 (m, 5 H), 1.84–2.04 (m, 6 H), 2.30 (ddd, J = 5.2, 14.2, 14.3 Hz, 1 H), 2.45–2.62 (m, 2 H), 2.74 (dq, J = 2.0, 4.1, 14.7 Hz, 1 H, 6 α -H), 4.73 (s, 1 H, 4-SH), 5.09 (br s, 1 H, NH); ^{13}C NMR (CDCl_3) δ 192.2, 171.6, 161.1, 128.6, 57.5, 55.6, 54.0, 51.1, 49.6, 40.2, 38.5, 35.2, 34.6, 33.4, 31.5, 31.2, 29.1 (3C), 24.3, 23.3, 21.1, 17.4, 13.1; EI-MS m/z (relative intensity) 403 (M^+ , 100), 388 (4), 329 (4),

9 245

304 (16), 276 (16), 220 (8), 147 (17), 128 (12), 81 (16), 72 (18); HRMS calcd for $C_{24}H_{34}O_2N_2S$, 403.2545, found 403.2541. Anal. ($C_{24}H_{34}O_2N_2S$) C, H, N, S.

N-(1,1-Dimethylethyl)-4-chloro-3-oxo-4-androstene-17 β -carboxamide (3f). *N,N*-(1,1-Dimethylethyl)-3-oxo-4-androstene-17 β -carboxamide (0.6 g, 1.62 mmol) was dissolved in dry pyridine (10 mL). Freshly distilled sulfuric chloride (0.56 g, 4.04 mmol) was added dropwise to the above agitating mixture at 20–25 °C over a period of 5 min. After 1 h, the reaction mixture was poured into water and extracted with diethyl ether (3 $\times$ 50 mL). The organic layer was washed with 10% aqueous HCl, 5% aqueous Na_2CO_3 , and brine. Drying and removal of the solvent gave the crude product which was purified by silica gel flash column chromatography (CH_2Cl_2 : C_6H_6 :EtOAc; 50:50:2 to 80:40:3) to give the product 3f (0.51 g, 77% yield): mp 153–155 °C; IR (KBr, cm^{-1}) 3390 (sh), 2966, 2852, 1699, 1668, 1583, 1525, 1451, 1390, 1362, 1292, 1252, 1224, 1185, 1115, 952, 812; ^1H NMR ($CDCl_3$) δ 0.73 (s, 3 H, 18- CH_3), 0.88 (t, J = 6.9, 7.0 Hz, 1 H), 0.94–1.13 (m, 8 H), 1.28 (s, 3 H, 19- CH_3), 1.24–1.26 (m, 1 H), 1.35 (s, 9 H, 3 CH_3), 1.43 (ddd, J = 3.5, 14.7, 14.6 Hz, 1 H), 1.52–1.63 (m, 3 H), 1.66–1.79 (m, 3 H), 1.90–2.06 (m, 4 H), 2.17 (ddd, J = 5.1, 14.5, 14.5 Hz, 2 H), 2.57 (dd, J = 4.5, 7.1 Hz, 2 H), 2.74 (dt, J = 3.3, 3.4, 14.6 Hz, 1 H, 6 α -H), 5.08 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$) δ 190.6, 171.6, 164.5, 127.4, 57.5, 55.5, 54.0, 51.1, 49.5, 41.4, 38.4, 35.2, 34.5, 34.1, 34.0, 31.1, 29.1 (3C), 24.3, 23.3, 21.1, 17.8, 13.1; EI-MS m/z (relative intensity) 405 (M^+ , 100), 390 (16), 369 (25), 350 (11), 289 (22), 252 (12), 227 (11), 141 (34), 128 (60), 115 (38), 93 (41), 81 (36), 72 (64); HRMS calcd for $C_{24}H_{32}O_2N_2Cl$, 405.2434, found 405.2405. Anal. ($C_{24}H_{32}O_2N_2Cl$) C, H, N, Cl.

N-(1,1-Dimethylethyl)-4-bromo-3-oxo-4-androstene-17 β -carboxamide (3g). Aqueous 40% HBr (10 mL) was added to a solution of *N,N*-(1,1-dimethylethyl)-3-oxo-4,5-epoxyandrostene-17 β -carboxamide (1.0 g, 2.59 mmol) in chloroform (100 mL) and glacial acetic acid (10 mL). After stirring for 16 h at room temperature under argon, the mixture was diluted with water (120 mL) and extracted with chloroform (3 $\times$ 100 mL). Drying and removal of the solvent gave an oil which was purified by silica gel flash column chromatography (CH_2Cl_2 : C_6H_6 :EtOAc; 50:50:2.5 to 60:40:2.5) to give the product 3g (0.81 g, 69% yield): mp 167–169 °C; IR (KBr, cm^{-1}) 3438 (sh), 3390, 2966, 2846, 1687, 1670, 1572, 1504, 1451, 1392, 1364, 1293, 1253, 1227, 1105, 948, 810; ^1H NMR ($CDCl_3$) δ 0.73 (s, 3 H, 18- CH_3), 0.86–1.121 (m, 4 H), 1.24 (s, 3 H, 19- CH_3), 1.29 (dd, J = 4.8, 4.9 Hz, 1 H), 1.35 (s, 9 H, 3 CH_3), 1.44 (ddd, J = 3.7, 13.0, 12.9 Hz, 1 H), 1.54–1.62 (m, 3 H), 1.66–1.80 (m, 3 H), 1.89–2.07 (m, 4 H), 2.17 (dd, J = 10.4, 10.7 Hz, 1 H), 2.26 (ddd, J = 5.2, 14.4, 14.5 Hz, 1 H), 2.56 (ddd, J = 4.6, 14.8, 14.8 Hz, 1 H), 2.62 (ddd, J = 3.9, 6.3, 11.1 Hz, 1 H), 3.27 (dt, J = 3.3, 3.6, 14.5 Hz, 1 H, 6 α -H); ^{13}C NMR ($CDCl_3$) δ 190.6, 171.6, 167.8, 121.9, 57.5, 55.5, 54.0, 51.1, 43.5, 42.5, 38.4, 35.8, 34.6, 34.0, 32.8, 31.2, 29.1 (3C), 24.3, 23.3, 21.2, 17.9, 13.1; EI-MS m/z (relative intensity) 451 (M^+ + 2, 67), 449 (M^+ , 68), 436 (7), 434 (8), 395 (7), 397 (7), 370 (100), 297 (22), 289 (32), 256 (11), 173 (23), 147 (32), 128 (37), 91 (47), 81 (35), 79 (35), 72 (53); HRMS calcd for $C_{24}H_{30}O_2N_2Br$, 449.1930, found 449.1905. Anal. ($C_{24}H_{30}O_2N_2Br$) C, H, N, Br.

Evaluation of the Inhibition of Human 5 α -Reductase (Types I and II). The measurements of *in vitro* inhibitory activity of compounds 3d–g on human 5 α -reductase (types I and II) were carried out according to the following procedures.

Type I 5 α -Reductase.^{2b} A total of 293 cells (ATCC CRL 1573) were transfected with the human type I 5 α -reductase cDNA and were used as the source of type I 5 α -reductase. After the transfection, cells were homogenized for the *in vitro* assay. Compounds to be tested were dissolved in ethanol and diluted with 50 mM Tris-HCl buffer containing 20% glycerol and 1 mM EDTA at pH 7.5. Inhibitors were first screened at two concentrations for 5 α -reductase inhibitory activity: 1 and 0.1 μ M. Compounds showing 50% or more inhibition at the 1 μ M concentration were subsequently tested at 12 concentrations ranging from 0.1 to 1000 nM for the measurement of the IC_{50} value. The indicated compound, 1 nM [^3H]androstenedione, 500 μ M NADPH, and the cell homogenate were added

to the sample tubes to a final volume of 1 mL. Following the 60-min incubation at 37 °C, the media were extracted twice with ether after the addition of 25 μ g each of nonradioactive steroid carriers (androstenedione and androstenedione). Steroids were separated by TLC, and the radioactivity was counted. Results are expressed as the amount of androstenedione produced as a percentage of control values.

Type II 5 α -Reductase.^{2a,b} SW-13 cells (ATCC HTB81) were transfected with the human 5 α -reductase type II cDNA^{2a} and were used as the source of type II 5 α -reductase. After transfection, cells were homogenized for the *in vitro* assay. Compounds to be tested were dissolved in ethanol and diluted with 50 mM Tris-HCl buffer containing 20% glycerol and 1 mM EDTA at pH 7.5. Inhibitors were first screened at two concentrations for 5 α -reductase inhibitory activity: 1 and 0.1 μ M. Compounds showing 50% or more inhibition at the 1 μ M concentration were subsequently tested at 12 concentrations ranging from 0.1 to 1000 nM for the measurement of the IC_{50} value. The indicated compound, 5 nM [^3H]androstenedione, 500 μ M NADPH, and the cell homogenate were added to the sample tubes to a final volume of 1 mL. Following a 60-min incubation at 37 °C, the media were extracted twice with ether after the addition of 25 μ g each of nonradioactive steroid carriers (androstenedione and androstenedione). Steroid were separated by TLC, and the radioactivity was counted. Results are expressed as the amount of androstenedione produced as a percentage of control values.

Inhibition of the Proliferation of Androgen-Sensitive Shionogi Cells. An androgen-sensitive cell line (clone SEM-107) derived from Shionogi mouse mammary carcinoma cells²¹ was used at passage 23. Cells were routinely grown as described previously.²² For the measurement of cell growth and sensitivity to anti-steroids, cells were plated at a density of 17400 cells/mL in minimal essential medium (MEM) supplemented with 2% dextran-coated charcoal-treated fetal calf serum, 1% nonessential amino acids, 10 IU/mL penicillin and 50 μ g/mL streptomycin. Steroids and anti-steroids were dissolved in ethanol, and stock solutions were prepared to yield a final ethanol concentration of less than 0.01% in the culture medium. Twenty-four hours after plating, medium was changed and the indicated concentrations of anti-steroids and DHT were added to triplicate dishes. Cells were grown for 13 days with medium changes every 3–4 days. Cells were then fixed in methanol, and their number was assessed by measurement of DNA content by a modification²³ of the method of Fiezer-Szafarz.²⁴ Dose-response curves and IC_{50} values were calculated using a weighted iterative nonlinear least squares regression.²⁵ Results are presented as means $\pm$ SEM of triplicate measurements.

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7/25 - (C) FILE HCAPLUS

STN CA Caesar accession number : 1181

XP-002187570

AN - 1995:423802 HCAPLUS

DN - 123:102007

TI - Relationship between structure and activity of 5.alpha.-
reductase inhibitors

IN - Guarna, A.; Marrucci, A.; Danza, G.; Serio, M.

CS - Department of Organic Chemistry "Ugo Schiff", Firenze, I-50121,
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SO - Int. Congr. Ser. (1994), 1064 (Sex Hormones and Antihormones in
Endocrine Dependent Pathology), 93-108
CODEN: EXMDA4; ISSN: 0531-5131

DT - Journal

LA - English

AB - The enzyme steroid 5.alpha.- reductase (E.C.1.3.99.5)
(5.alpha.-R) is a system of two NADPH-dependent isoenzymes which
catalyzes the conversion of testosterone (T) to
dihydrotestosterone (DHT) in many
androgen-sensitive cells. The prodn. of DHT is related to
several human endocrine diseases such as benign
prostatic hyperplasia (BPH), prostatic
cancer, baldness, acne, alopecia in men and hirsutism in women.
Thus, the blockade of the DHT formation without
deprivation of T, by using selective 5.alpha.-R inhibitors, is an
important target in pharmaceutical and medical research. A mol.
modeling study has been developed to establish the indispensable
mol. features to inhibit the human prostatic enzyme 5.alpha.-R. The
active site model was obtained using the "active analog approach";
by taking the differences between the combined vols. of a set of
inactive mols. and the combined vols. of a set of active mols. The
resulting three-dimensional area represents a part of the space
occupied by the enzyme. This approach is useful to predict the
inhibitory activity of steroidal compds. towards 5.alpha.-R because
the values of intersection with the cavity model are inversely
correlated with the inhibitory potency of the compds. Therefore
chem. syntheses can be directed towards the compds. which showed a
good structure-activity relation.

IT - 1093-58-9

RL: BAC (Biological activity or effector, except adverse); BIOL
(Biological study)

(relationship between structure and activity of 5.alpha.-
reductase inhibitors)

RN - 1093-58-9 HCAPLUS

CN - Androst-4-en-3-one, 4-chloro-17-hydroxy-, (17.beta.)- (9CI) (CA
INDEX NAME)

Absolute stereochemistry.

[--00000047]

IT ***9081-34-9*** , Steroid 5.alpha.- ***reductase***

RL: BPR (Biological process); BIOL (Biological study); PROC
(Process)

(relationship between structure and activity of 5.alpha.-
reductase inhibitors)

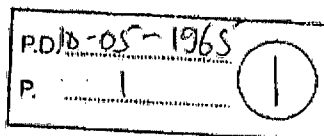
RN 9081-34-9 HCAPLUS

CN Reductase, steroid 5.alpha.- (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

04 248
12

Page:



Wittig reaction of steroid ketones. Kurt Barnikol-Oettler, Rudolf Zepfer, and Karl Heller. *J. Prakt. Chem.* 27(1-2), 18-33 (1965)(Ger). The reactions of several 6- and 17-oxo steroids with $(\text{Ph}_3\text{PMe})\text{Br}$ and PhLi or NaNH_2 are described; CO groups not to be olefinated were protected by ketalization. The 6-oxo steroids of the 5 α -series were converted in this manner in good yields into the 6-methylene analogs. The olefination of the 6-position in the 5 β -steroids, however, was more difficult because of steric effects. 5 β -Androstane-3,6 α ,17 β -triol 17-acetate (5 g.) in 50 cc. 80% AcOH treated at 8-10° for 18 min. with 3.8 g. CrO_3 in 40 cc. 80% AcOH and kept 3.5 hrs. at 15° yielded 2.39 g. 5 β -androstan-17 β -ol-3,6-dione 17-acetate (I), m. 160-4° and 101.5-63° (Me_2CO), $[\alpha]_D -114^\circ$ (all rotations were measured in CHCl_3). I (1.3 g.) in 13 cc. 2-ethyl-2-methyl-1,3-dioxolane (II) treated at 100° with 50.7 mg. $p\text{-MeC}_6\text{H}_4\text{SO}_3\text{H}$ and cooled after 5 min. to below 0° yielded 1.18 g. 3-ethylene ketal (III) of I, m. 106-6.8° (MeOH), $[\alpha]_D -55.7^\circ$. III (900 mg.), 900 mg. K_2CO_3 , 18 cc. MeOH, and 3.7 cc. H_2O refluxed 1.5 hrs. gave 629 mg. 3-ethylene ketal (IV) of 5 α -androstan-17 β -ol-3,6-dione (V), m. 188.2-8.7° (MeOH), $[\alpha]_D -22^\circ$. I (393 mg.) in 13 cc. MeOH refluxed 2 hrs. with 400 mg. K_2CO_3 and 1.2 cc. H_2O gave 247 mg. V, m. 234-6° (Me_2CO), $[\alpha]_D -8^\circ$ (CHCl_3). V (800 mg.) in 17 cc. II heated 4 min. at 100° with 36 mg. $p\text{-MeC}_6\text{H}_4\text{SO}_3\text{H}$ gave 518 mg. IV, m. 188-8.7° (MeOH), $[\alpha]_D -22^\circ$, and about 80 mg. mixt. of V, IV, and the diketal. $(\text{Ph}_3\text{PMe})\text{Br}$ (13 g.) and 144 cc. dry Et_2O treated with stirring at room temp. with 72 cc. Et_2O contg. 3 g. PhLi and then with 2.6 g. IV in 14 cc. dry tetrahydrofuran (THF) and 72 cc. dry Et_2O , stirred 4 hrs. at room temp., and kept overnight, 290 cc. Et_2O replaced by 220 cc. THF, and refluxed 1 hr., and the product chromatographed on 150 g. silica gel yielded 1.92 g. 3-ethylene ketal (VI) of 6-methylene-5 α -androstan-17 β -ol-3-one (VII), m. 181.5-82° (MeOH), $[\alpha]_D -16.6^\circ$. VI (1 g.) in 70 cc. Me_2CO refluxed 2 hrs. with 8.6 cc. 2N H_2SO_4 gave 723 mg. VII, m. 196-7.2° (Me_2CO), $[\alpha]_D -25.5^\circ$; acetate (VIII) m. 187.5-8.5°, $[\alpha]_D -27.5^\circ$. VIII (523 mg.) in 5.85 cc. $\text{C}_6\text{H}_5\text{N}$ stirred 4 hrs. at room temp. with 406 mg. OsO_4 , treated with 720 mg. NaHSO_4 in 11.6 cc. H_2O and 7.8 cc. $\text{C}_6\text{H}_5\text{N}$, and stirred 0.5 hr. gave 445.6 mg. 6 β -hydroxymethyl-5 α -androstan-6 α ,17 β -diol-3-one (IX), m. 174-8.2° (Me_2CO), $[\alpha]_D -1^\circ$. IX with $\text{Ac}_2\text{O}-\text{C}_6\text{H}_5\text{N}$ gave 6 β -acetoxy-methyl-17 α -acetoxy-5 α -androstan-6 α -ol-3-one, m. 142.5-3.5°, $[\alpha]_D 6^\circ$. Diacetate (30 g.) of 24,24-diphenyl-5 β -chol-23-ene-3,6 α -diol (X) in 600 cc. MeOH refluxed 2 hrs. with 30 g. K_2CO_3 in 90 cc. H_2O gave 32.5 g. X, m. 180-6°. X (25.5 g.) in 400 cc. $\text{C}_6\text{H}_5\text{N}$ treated during 2 hrs. with stirring at room temp. with 10.45 g. CrO_3 , stirred 1 hr., and kept overnight yielded 14.5 g. mixt., m. 108-13° (MeOH); an 8-g. portion chromatographed on 200 g. silica gel gave 1.8 g. 24,24-diphenyl-5 β -chol-23-ene-3,6-dione, m. 148-64°, $[\alpha]_D -33.8^\circ$, and 3.2 g. 24,24-diphenyl-5 β -chol-23-en-3 α -ol-6-one (XI), m. 145-8°, $[\alpha]_D -6^\circ$. XI (983 mg.) in 30 cc. MeOH refluxed 2 hrs. with 1 g. K_2CO_3 in 3 cc. H_2O gave 514 mg. 5 α -epimer of XI, m. 201-5° (MeOH- Me_2CO), $[\alpha]_D 27.5^\circ$. $(\text{Ph}_3\text{PMe})\text{Br}$ (13.35 g.) added during 1 hr. under argon to NaNH_2 prep'd. from 940 mg. Na and 170 cc. liquid NH_3 , the NH_3 replaced by 60 cc. dry Et_2O , and the mixt. dild. with an addnl. 60 cc. Et_2O , treated with 2.5 g. 4-chloro-4-androstene-3,17-dione 3-ethylene ketal in 90 cc. dry THF, stirred 2 hrs. at room temp., kept overnight, coned. during 15 min. to remove 150 cc. solvent, dild. with 60 cc. dry THF, and refluxed 15 min. gave 0.37 g. unreacted ketal, m. 230-4°, and 1.65 g. 17-methylene-4-chloro-4-androsten-3-one 3-ethylene ketal (XII), m. 181.5-83° (Me_2CO), $[\alpha]_D 133^\circ$. XII (1 g.) in 60 cc. Me_2CO refluxed 2 hrs. with 8.5 cc. 2N H_2SO_4 gave 710 mg. 4-chloro-17-methylene-4-androsten-3-one, m. 138.4-40° (MeOH), $[\alpha]_D 160^\circ$. 17-Methylene-5-androsten-3-one 3-ethylene ketal (1.48 g.) in 90 cc. Me_2CO refluxed 2 hrs. with 2N H_2SO_4 gave 84% 17-methylene-4-androsten-3-one, m. 133.5-35°, $[\alpha]_D 125^\circ$.

F. W. Hoffmann