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SYNTHESIS AND EVALUATION OF 3-CARBOXYMETHYL STEROIDS AS INHIBITORS OF HUMAN PROSTATIC STEROID 5α-REDUCTASE

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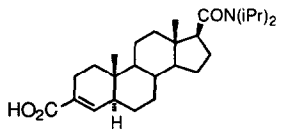
Abstract: A series of 3-carboxymethyl steroids has been prepared and assayed *in vitro* as inhibitors of steroid 5α-reductase isolated from human prostatic tissue. Potent inhibition was observed with analogs from both androstene and estratriene families. Phosphonic and sulfonic acid analogs were also prepared and found to lack significant inhibitory activity.

Dihydrotestosterone (DHT), a metabolite of the major circulating androgen testosterone, produced by the NAPDH-dependent-enzyme steroid 5α-reductase, is recognized to be the principal mediator of several androgen-related conditions. The most notable of these is benign prostatic hyperplasia (BPH), an ailment afflicting a remarkably large percentage of aging males, which is often accompanied by the discomforting symptoms of urinary dysfunction.[1] In addition, elevated levels of DHT have been implicated in the etiology of some prostatic cancers[2] and several skin disorders including acne,[3] male pattern baldness,[4] and hirsutism.[5] As a result of the elucidation of these physiological roles of DHT within the past twenty years, an active search has ensued for potent inhibitors of steroid 5α-reductase as potential therapeutics.[1] In 1992 the first such inhibitor (finasteride[6]) was approved in the U.S. for treatment of BPH. While the steady state kinetic mechanism of steroid 5α-reductase has been well characterized,[7] only recently has molecular characterization revealed the existence of two isozymes encoded by distinct genes.[8] Elucidating the tissue expression and physiological roles of these enzyme isoforms remain areas of intense research.

As part of our ongoing effort to discover novel inhibitors with potential pharmaceutical utility, we have previously reported on the synthesis and characterization of several classes of potent inhibitors of human prostatic steroid 5α-reductase activity exemplified by compounds **1**, **2**, and **3**.[9-14]



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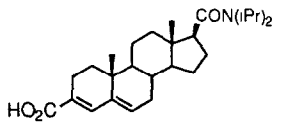

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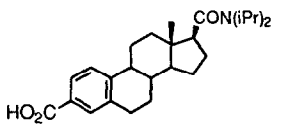


3

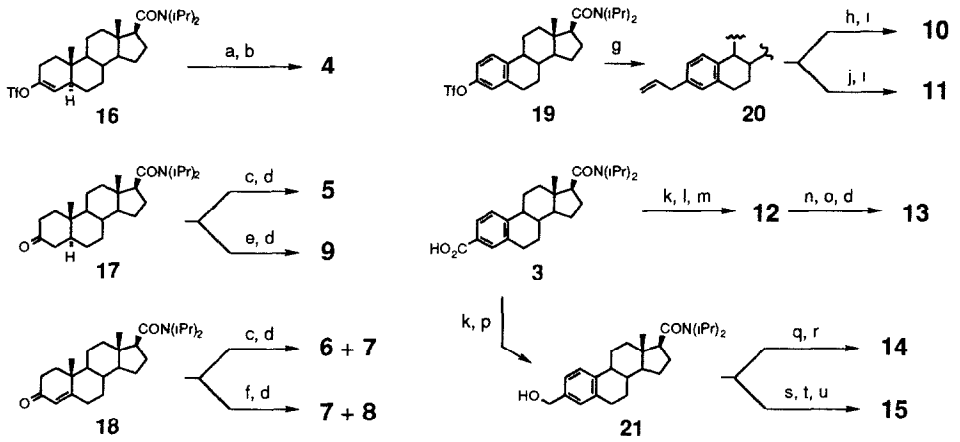
The first of these compound classes (steroidal acrylates, e.g **1** and **2**) derived from consideration of the chemical mechanism of the enzyme and were designed as potential mimics of the putative enolate intermediate -- the key feature being the use of a carboxylic acid functional group as a stable, anionic surrogate for the enolate oxyanion.[9,10] Subsequently, we demonstrated inhibitory activity by the analogous estrone-derived A-ring aromatic carboxylic acids (e.g. **3**)[11] and later by phosphonic,[12] phosphinic,[12] and sulfonic acid analogs.[13] We proposed that the anionic acid functionality coordinates to an active site electrophile(s) which may be present to activate the substrate enone for hydride transfer, to stabilize the resulting enolate, and/or to transfer a proton to the steroidal substrate.[15]

The notion that the C-3 carboxylate coordinates to an enzyme active site electrophile led us to synthesize homologated analogs of **1**, **2** and **3** which, through greater conformational freedom of the C-3 appendage, might allow for an enhanced interaction of this nucleophilic group with active site electrophiles.

Herein we report the synthesis and evaluation of several homologated analogs which exhibit a range of inhibitory potencies against human prostatic steroid 5α-reductase activity.

Chemistry. The synthesis of **4** (Table 1) was accomplished in two steps (Scheme 1) from the known enol triflate **16**[10] relying on a palladium-catalyzed cross-coupling of a Reformatsky reagent.[16] The isomeric Δ^2-β,γ-unsaturated acid **5** was prepared by reacting a Horner-Emmons reagent under equilibrating conditions (NaOEt, EtOH) with ketone **17**, whereas treatment of **17** with the same phosphonate reagent and NaH in DMF provided, after hydrolysis, the α,β-unsaturated acid **9**. Horner-Emmons reaction using enone **18** afforded a mixture of (*E*)-α,β,γ,δ-unsaturated dienyl and $\Delta^{3,5}$-β,γ,δ,ε-unsaturated esters which were separable after hydrolysis to yield **6** and **7**. Addition of *in situ*-generated lithium methoxyacetylide[17] to enone **18** followed by acid treatment and ester hydrolysis led to the isolation of the isomeric (*Z*)-conjugated dienyl acid **8** as a separable mixture with **7**.

Scheme 1.[19]



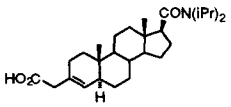
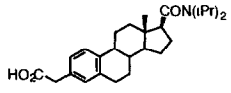
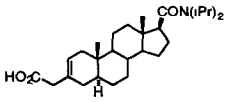
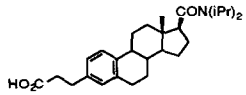
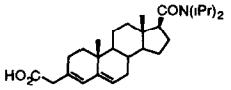
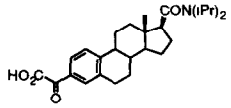
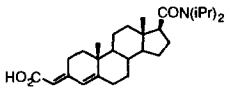
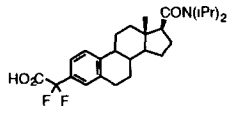
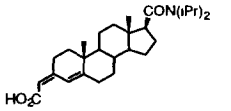
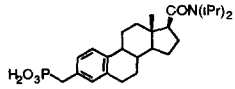
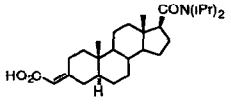
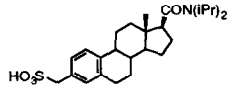
a) $(Ph_3P)_2PdCl_2$, DIBAL, 0°, then $BrZnCH_2CO_2{}^tBu$, r.t., b) LiOH, H_2O, MeOH, THF, reflux; c) $(EtO)_2P(O)CH_2CO_2Me$, NaOEt, EtOH, reflux; d) K_2CO_3, MeOH, H_2O, reflux; e) $(EtO)_2P(O)CH_2CO_2Me$, NaH, DMF, reflux; f) $Br_2HCCHClOMe$, nBuLi, -78° to 0°; H_2SO_4; g) allylSnBu3, $(Ph_3P)_2PdCl_2$, LiCl, dioxane; h) O_3; Me_2S; i) Jones reagent, j) 9-BBN; H_2O_2, KOH; k) $SOCl_2$; l) NaCN, Bu_4NBr; m) conc. HCl; n) CH_2N_2, o) DAST; p) $NaBH_4$, q) $P(OEt)_3$; r) TMSCl, NaI; s) PBr_3; t) $(H_2N)_2CS$, NaOH; u) Cl_2, HOAc; K_2CO_3, H_2O, acetone.

One and two carbon homologated A-ring aryl acids **10** and **11** were prepared from aryl triflate **19** via the intermediacy of the allylstannane coupling product **20**.[18] Ozonolysis of **20** followed by Jones oxidation led to homolog **10** while hydroboration/oxidation led to the double homolog **11**. The α-keto and α,α-difluoro analogs of **10** were synthesized from the previously described[11] aryl acid **3**. Formation of the acyl cyanide followed by acid hydrolysis afforded the ketoacid **12**. Treatment of the methyl ester of **12** with diethylaminosulfur trifluoride (DAST) and subsequent hydrolysis provided difluoro acid **13**. Finally, preparation of the phosphonic and sulfonic acid analogs proceeded through the **3**-derived benzyl alcohol **21**. Arbuzov reaction followed by dealkylation with TMSI provided the benzyl phosphonic acid analog **14**. Conversion of alcohol **21** to the benzyl bromide followed by thiourea displacement, hydrolysis, and oxidation provided the corresponding sulfonic acid analog **15**.

Enzyme Inhibition. In spite of the greater steric demands of the C-3 appendage, the regioisomeric acrylate homologs **4** and **5** exhibited potent inhibition of human prostatic steroid 5α-reductase activity[20] (inhibition constants of 85 and 40 nM, respectively, Table 1), comparable to the parent acrylate **1** ($K_{i,app}$ = 30 nM) and its Δ^2 isomer (not shown, $K_{i,app}$ = 85 nM).[10] In contrast, the $\Delta^{3,5}$ dienyl acid **6** was a relatively weak inhibitor ($K_{i,app}$ = 450 nM) as compared to its parent **2** ($K_{i,app}$ = 7 nM).[10] While the original design rationale

was to provide greater conformational freedom for the carboxylate in the active site, compounds **7**, **8**, and **9** with exocyclic double bond configurations were readily accessible and allowed examination of two different but specific orientations of the carboxylate. However, these compounds were devoid of measurable inhibitory activity.

Table 1.

Compd No.	Structure	$K_{i,app}$ (nM)	Compd No.	Structure	$K_{i,app}$ (nM)
4		**85**	**10**		**20**
5		**40**	**11**		**290**
6		**450**	**12**		**150**
7		**>5000**	**13**		**40**
8		**>5000**	**14**		**740**
9		**>5000**	**15**		**>5000**

Homologs in the estrane series also demonstrated potent inhibition as exemplified by **10** which, with a $K_{i,app}$ = 20 nM, is equipotent with its parent, aryl carboxylate **3**.[11] Extending the connecting chain to two carbons (compound **11**) resulted in an inhibitory activity reduced by an order of magnitude. Substitution of the benzylic carbon with gem-difluoro and oxo groups (which not only lower the carboxylate pK_a but also act as potential hydrogen bond acceptors) resulted in a maintenance of activity in the former (**13**, $K_{i,app}$ = 40 nM) and a diminution of activity in the latter case (**12**, $K_{i,app}$ = 150 nM). Finally, extending this inhibitor design strategy to phosphonic and sulfonic acid analogs **14** and **15** resulted in a remarkable loss of activity ($K_{i,app}$ = 740 and >5000 nM, respectively) compared to their non-homologated parents[12,13] which were characterized by inhibition constants in the range of 20 to 50 nM.

In summary, inhibition of human prostatic steroid 5α-reductase has been demonstrated with a series of 3-carboxymethyl steroids. These compounds derive from previously described 3-carboxy steroid classes of inhibitors yet structure-activity relationships within the parent and homologated series do not entirely correspond.

References and Notes

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19. All new compounds were characterized by ^{1}H-NMR. Compounds **4-15** provided satisfactory elemental analyses and mass spectra. Melting points: **4**: 236-239°C, **5**: 270°C, **6**: 205-207°C, **7** 308°C, **8**: 215-217°C, **9**. foam, **10**: 125-127°C, **11**: 110-115°C, **12**: 251-253°C, **13**. 222-224°C, **14**: 190°C, **15** 275°C (as potassium salt).

    Regiochemical assignments for isomeric **4** and **5** are consistent with the maintenance of double bond position in the Pd-catalyzed coupling of **16** and the isomerization of the double bond of **5** into the thermodynamically more stable position under equilibrating conditions The downfield chemical shift for the vinylic proton of **5** relative to **4** is consistent with that observed in the analogous acrylates (e.g. **1**)[10]. Partial ^{1}H-NMR for **4**: δ 2.95 (s, 2H, HO_2C-C$\underline{H}_2$-), 5.2 (s, 1H, H-4); **5**: δ 3.0 (s, 2H, HO_2C-C$\underline{H}_2$-), 5.5 (d, J = 5.6 Hz, 1H, H-2). Structural assignment of **6** was consistent with nOe observed for H-6 and H_2-21 (HO_2C-C$\underline{H}_2$-) upon irradiation of H-4. Partial ^{1}H-NMR for **6**: δ 3.06 (s, 2H, HO_2C-C$\underline{H}_2$-), 5.40 (d, J = 3.0 Hz, 1H, H-6), 5.87 (s, 1H, H-4). Geometrical isomers **7** and **8** were assigned as *E* and *Z*, respectively, based on observed nOe between H-4 and H-21 of **7** and no such enhancements for **8**. The downfield shift for H-4 of **8** relative to that of **7** is consistent with a deshielding effect of the proximal carboxylate in **8**. Partial ^{1}H-NMR for **7**: δ 5.54 (s, 1H, H-4), 5.84 (s, 1H, H-21), **8**: δ 7.18 (s, 1H, H-4), 5.41 (s, 1H, H-21). Geometrical assignment for **9** was based on an observed nOe for H-4a upon irradiation of H-21 H-4a and H-4b were assigned by two-dimensional ^{1}H-NMR (COSY) correlations. Compound **9** partial ^{1}H-NMR: δ 1.93 (H-4a), 2 22 (H-4b), 5.62 (s, H-21).
20. Evaluation of steroid 5α-reductase activity was performed with microsomal human prostatic enzyme preparations as previously described.[9] Apparent inhibition constants ($K_{i,app}$) of test compounds were determined by the method of Dixon [21]
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