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# 3-Carboxy-20-Keto Steroids are Dual Uncompetitive Inhibitors of Human Steroid 5α-Reductase Types 1 and 2

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**Abstract**—Steroidal 3-carboxy-20-ketones have been prepared within two structural series, the androsta-3,5-dienes and the estra-1,3,5-trienes, as potential inhibitors of types 1 and 2 steroid 5α-reductase, the enzyme activity responsible for the final step in biosynthesis of dihydrotestosterone. These compounds are shown to be potent uncompetitive inhibitors of both human recombinant enzyme activities, defining a novel class of dual steroid 5α-reductase inhibitors. Copyright © 1996 Elsevier Science Ltd

## Introduction

The potent androgen 5α-dihydrotestosterone (DHT) has been implicated in the pathogenesis of several diseases such as benign prostatic hyperplasia (BPH), prostatic cancer,[1] male-pattern baldness,[2] acne vulgaris,[3] and hirsutism.[4] DHT is biosynthesized by reduction of testosterone (T), an enzymatic reaction catalysed by steroid 5α-reductase (SR).[5] It is now recognized that formation of DHT can be catalyzed by two forms of SR known as type 1 and type 2, which are encoded by unique genes and display differential developmental characteristics and tissue distributions.[6,7]

Identification of inhibitors of these enzymes for use in treatment of the above-mentioned disorders has been the objective of research efforts within several laboratories. The best characterized inhibitors are epristeride (**1**)[8,9] and finasteride (**2**).[10] Both are selective inhibitors of type 2 SR.[11,12] In clinical studies, both compounds have been shown to be effective at lowering serum DHT levels in man by as much as 50–80% relative to baseline values.[13,14] Although the reasons for incomplete suppression of plasma DHT are not clear, it can be proposed that residual DHT concentrations result from type 1 SR biosynthesis which may not be completely blocked by the type 2 selective inhibitors **1** or **2**. Other structural classes of SR inhibitors include the vinylogous aza-steroid represented by **3**[15] and nonsteroids as exemplified by benzoyl-benzophenone carboxylic acid (**4**),[16] indole carboxylic acid (**5**),[17,18] and benzoquinolinone (**6**)[19] (Fig. 1). Of these specific examples, **4** and **5** are selective for type 2 SR, **6** represents a selective type 1 inhibitor, while **3** has been reported as a dual inhibitor of both SR isoforms. The role of type 1 SR in normal physiology and human disease states is not fully understood.

Based on the hypothesis that inhibition of both SR isozymes would be more effective in lowering systemic DHT compared with isotype selective inhibitors, and consequently may be more efficatious in controlling diseases related to increased levels of this androgen, our research efforts were refocused toward identification of compounds that would inhibit both SR isoforms.

## Results and Discussion

Efforts toward the objective of identifying dual SR isoform inhibitors began with a thorough evaluation of the uncompetitive SR inhibitors, exemplified by **1**, that had been the subject of our earlier research.[12,20,21] The results from these experiments indicated that none of the previously characterized 3-carboxy steroidal inhibitors which were substituted with 17β-carboxamides demonstrated significant inhibitory activity on type 1 human SR.[22] For reference, epristeride (**1**) was found

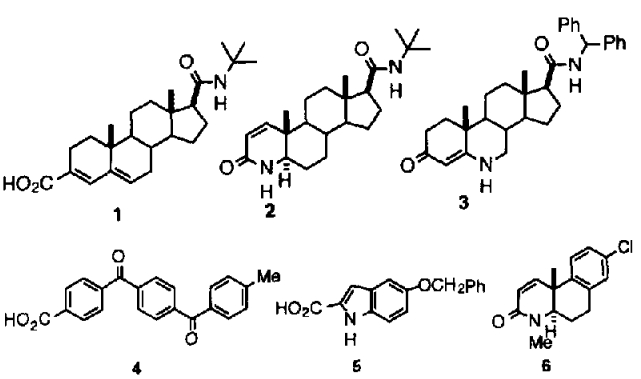


**Figure 1.** Structural motifs for known steroid 5α-reductase inhibitors.

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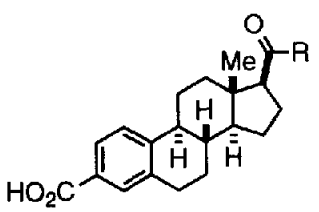
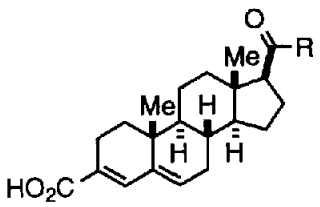
to have approximate values for $K_{i,app}$ of 0.12 nM and 412 nM against human, recombinant isoforms 2 and 1, respectively.[12,20] Results with *tert*-butyl carboxamide **7** (Table 1), the aromatic A-ring analogue of **1**, showed a similar selectivity for type 2 SR.[20]

An early study with alternative 3-keto-4-ene-steroids suggested progesterone to be a significantly better substrate of type 1 SR than testosterone.[7] The increased catalytic efficiency of type 1 SR with progesterone ($V/K_m$ = 1230 pmol/min/mg/μM) versus testosterone ($V/K_m$ = 40–60 pmol/min/mg/μM) was found to predominantly result from a significant decrease in the Michaelis constant of progesterone ($K_m$ = 0.3 μM) as compared to that for testosterone ($K_m$ = 3.5–5.2 μM).[20] This observation suggested that introduction of 20-keto-substituents, conceptually derived from the progesterone side-chain, onto known steroidal inhibitor scaffolds displaying selectivity for type 2, would improve type 1 inhibitory potency. At the same time, inhibitory potency for type 2 SR should be maintained since the catalytic properties of progesterone and testosterone with this isoform were comparable.[20] Thus, with the goal of increasing the isoform 1 inhibitory activity, alternative substitutions at the 17 position incorporating the 20-keto functionality were investigated within the two steroidal frameworks, androsta-3,5-diene-3-carboxylic acids and estra-1,3,5-triene-3-carboxylic acids. Both structural motifs have been previously characterized as uncompetitive SR inhibitors, resulting from the preferential binding of steroidal inhibitor and $NADP^+$ to enzyme.[8,12,23] Inhibitory poten-

**Table 1.** 3-Carboxy-20-keto steroidal inhibitors of steroid 5α-reductase types 1 and 2




A




B

| R | Compd | Series | Isoform 1 $K_{i,app}$ (nM)[a] | Isoform 2 $K_{i,app}$ (nM) | Ratio of inhibition potencies for type 1/type 2[b] |
|---|---|---|---|---|---|
| N(H)-*t*Bu | **1** | B | 412 | 0.12 | 3433 |
| N(H)-*t*-Bu | **7** | A | 1600 | 0.4 | 4000 |
| Ph | **8** | A | 60 | 4–8 | 10 |
| Ph | **9** | B | 61 | 1–8 | 14 |
| 4-F-Ph | **10** | A | 97 | 2 | 49 |
| 4-F-Ph | **11** | B | 85 | 2 | 43 |
| 4-$F_3$C-Ph | **12** | A | 55 | 3 | 18 |
| 3,5-diF-Ph | **13** | A | 45 | 1 | 45 |
| 4-$MeSO_2$Ph | **14** | A | 80 | 8 | 10 |
| 4-MeSPh | **15** | A | 30 | 1 | 30 |
| 4-MeOPh | **16** | A | 40 | <2 | ~20 |
| 4-PhOPh | **17** | A | 52 | 4 | 13 |
| 4-Ph-Ph | **18** | A | 50 | 8 | 6.3 |
| 1-Naphthyl | **19** | A | 110 | 6 | 18 |
| Me | **20** | A | 35 | 20–30 | 1.4 |
| *i*-Pr | **21** | A | 30 | 1 | 30 |
| *i*-Pr | **22** | B | 55 | 3 | 18 |
| *i*-Bu | **23** | A | 54 | 2–4 | 18 |
| *i*-Bu | **24** | B | 38 | 1 | 38 |
| $CH_2$Ph | **25** | A | 30 | 1 | 30 |
| $CH_2$Ph | **26** | B | 10–30 | 0.2–0.4 | 67 |
| $CH_2CH_2$Ph | **27** | A | 7 | 1–2 | 4.7 |
| $CH_2CH_2$Ph | **28** | B | 4–6 | 1–2 | 3.3 |
| $CH_2CH_2$-4-MeOPh | **29** | A | 3 | 1 | 3 |
| $CH_2CH_2$-4-MeOPh | **30** | B | 3 | 2 | 1.5 |
| $CH_2CH_2$-4-F-Ph | **31** | A | 3 | 3 | 1 |
| $CH_2CH_2$-4-F-Ph | **32** | B | 7 | 6 | 1.2 |

[a]Type 1 and type 2 SR activities were determined at pH 7.5 and 5.0, respectively. All results were obtained with recombinant human enzymes expressed in CHO cells as described.[12,20] Standard error for all experimental values were less than 20%. Ranges indicate the results of multiple determinations.

[b]For those compounds whose $K_{i,app}$ values are presented as a range, the ratios of inhibition potencies for isoform 1/isoform 2 were based on their calculated mid-points.

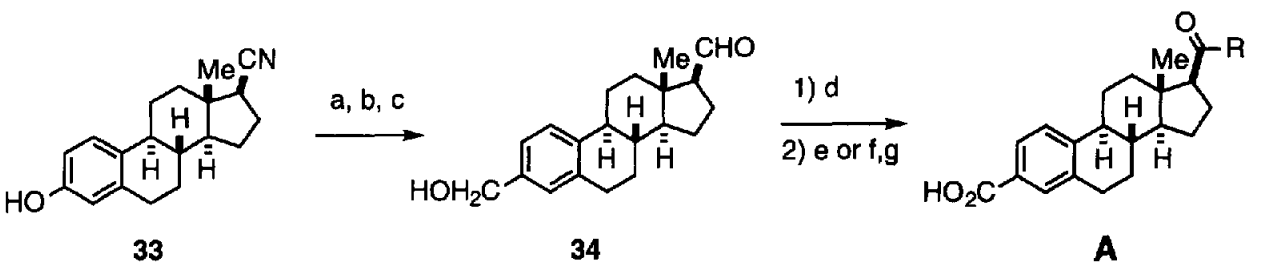
cies of selected aryl, alkyl, and substituted alkyl steroidal ketones with recombinant human SR isoforms 1 and 2 are shown in Table 1.

Greater inhibitory potency on type 1 human SR was observed with all ketones versus the 17β-carboxamides represented by **1** and **7**. All the 20-keto compounds presented in Table 1 are shown to maintain excellent inhibitory potency on type 2 enzyme activity. Within the series of alkyl ketones (**20–24**), only slight differences in type 1 inhibitory potencies were observed. In contrast, substitution of the alkyl chain with aromatic groups (**25–32**) significantly enhanced potency toward the type 1 activity. This resulted in identification of the aryl substituted ethyl ketones (**27–32**) as the most potent dual inhibitors within these series. Marginal differences in inhibitory potencies were observed upon substitution of the side-chain aromatic ring within the aryl ketones (**8–19**). In addition, the relative potencies of compounds in the dienyl 'B' series were not significantly different from those for analogous ketones in the aromatic A-ring 'A' series, as exemplified by compound pairs **8/9**, **10/11**, **23/24**, **25/26**, **27/28**, and **31/32**.

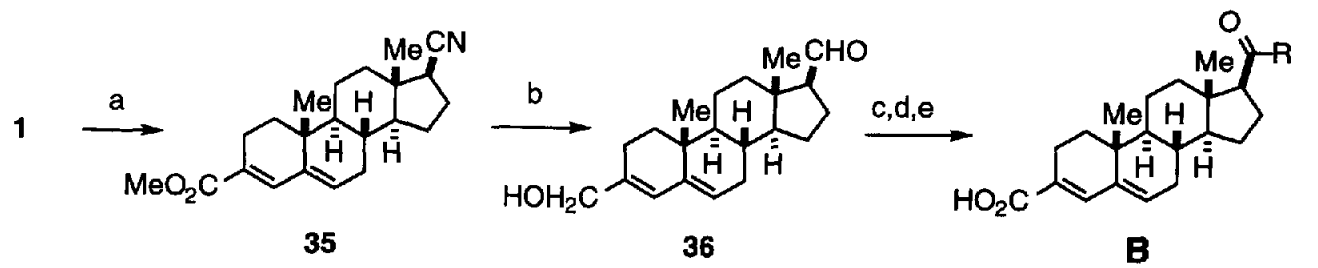
Previous kinetic analyses have shown that the 17β-carboxamide substituted dienyl acid steroids (series 'B') or aromatic A-ring steroids (series 'A') are uncompetitive inhibitors versus steroid substrate of enzyme derived from human[8] and animal tissues.[24,25] More recently, epristeride (**1**) has been shown to display uncompetitive kinetic patterns with both recombinant human SR types 1 and 2.[12] These kinetic patterns have been interpreted to result from the preferential association of the steoridal 3-carboxylic acids in a complex with enzyme and oxidized cofactor, E-NADP$^+$. Comparable results have now been shown for the dual-isoform inhibitors presented in Table 1. For example, the potent dual isoenzyme inhibitor **27**, possessing a phenethylketone side-chain substituent, is an uncompetitive inhibitor (data not shown) of both recombinant human SR enzymes, with values for $K_{ii}$ of $5 \pm 1$ nM and $0.3 \pm 0.1$ nM on isoforms 1 and 2, respectively. Hence, side-chain substitution leading to more potent type 1 interaction does not change the kinetic mechanism of inhibition.

Increasing the potency of steroidal-based inhibitors toward type 1 SR by modification of the 17β-substituent also has been demonstrated within the 4-aza (e.g., **2**) and the 6-aza (e.g., **3**) structural series.[11,15] With the aza structural classes, however, inhibition results from their preferential association to enzyme in the presence of reduced cofactor, NADPH. These compounds also appear to be time-dependent inhibitors of types 1 and 2 SR, although the exact molecular events giving rise to these observations have not been fully elucidated.

Based on the clinical effects in the lowering of systemic levels of DHT by finasteride and epristeride, it has been proposed that an inhibitor that is more effective on both SR enzymes may prove to have advantages in the treatment of DHT dependent disease states. Toward this objective, we have conducted studies to evaluate the effects on plasma DHT levels in the *Cynomolgus* monkey with several of the more potent dual inhibitors presented in Table 1, including **25**, **26**, **27**, **28**, **31**, and **32**. Since the inhibitory effects in vitro




**Scheme 1.** Synthesis of 20-keto-estra-1,3,5-trienes. (a) $PhNTf_2$, NaH, THF; (b) cat. $Pd(OAc)_2$, CO, dppf, DMF, $Et_3N$, MeOH; (c) DIBAl, PhMe; (d) RMgX, THF; (e) Jones reagent, acetone; (f) cat. TPAP, NMO, 4 Å sieves, $CH_2Cl_2$; (g) $NaClO_2$, THF, $H_2O$, 2-methyl-2-butene.




**Scheme 2.** Synthesis of 20-keto- androsta-3,5-dienes. (a) $POCl_3$, PhMe; MeOH; (b) DIBAl, PhMe; (c) RMgX, THF; (d) cat. TPAP, NMO, 4 Å sieves, $CH_2Cl_2$; (e) $NaClO_2$, THF, $H_2O$, 2-methyl-2-butene.

of monkey SR isoenzymes parallels that found for the human counterparts,[12,20] this species is thought to provide a preferred model for response of systemic DHT to inhibitors. Each of these dual inhibitors has been shown to cause significant and prolonged suppression of plasma DHT in the *Cynomolgus* monkey upon single dose oral administration. Pharmacokinetic parameters (e.g., oral bioavailability, plasma half-life and rates of clearance) of these compounds are being evaluated in order to better understand the interrelationships between plasma DHT suppression in the monkey to the compounds' inherent inhibitory potencies on the two SR isoenzymes and their systemic exposures. Ultimately, however, the demonstration of improved effect in relief of symptoms associated with disease states in which DHT is a contributing agent must await the results from future clinical trials in evaluation of selective and non-selective inhibitors of the SR isoenzymes.

## Experimental

### Chemistry

Ketone analogues comprised of the estra-1,3,5-triene skeleton (referred to as the 'A' series) were synthesized as shown in Scheme 1. Nitrile **33** was converted to aldehyde **34** by triflation of the alcohol, palladium-mediated alkoxy carbonylation of the triflate, and DIBAl reduction. Addition of a Grignard reagent to the aldehyde and Jones oxidation of alcohols, or a 2 step TPAP oxidation,[26] followed by $NaClO_2$ oxidation[27] gave the desired compounds in series 'A' (Scheme 1).

Ketones comprised of the androsta-3,5-diene skeleton (referred to as the 'B' series) were prepared by using an analogous strategy starting with epristeride (**1**).[28] Nitrile **35** was made by converting the *tert*-butyl amide with $POCl_3$ using a procedure devised by Gribble.[29] DIBAl reduction of the nitrile and ester moieties gave aldehyde **36**, which was converted to the desired compounds by the addition of a Grignard reagent, and was subsequently followed by a two-step oxidation sequence as described above (Scheme 2) to provide the 'B' series compounds. Alternatively, a literature procedure utilizing a 2-pyridyl-thio ester intermediate was employed as described in preparation of series 'B' compounds.[30]

### Inhibitor evaluation

Type 1 and type 2 SR activities were determined at pH 7.5 and 5.0, respectively. All results were obtained with recombinant human enzymes expressed in CHO cells as previously has been described.[12,20]

## Acknowledgments

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