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Steroidal nitrone inhibitors of 5α-reductase

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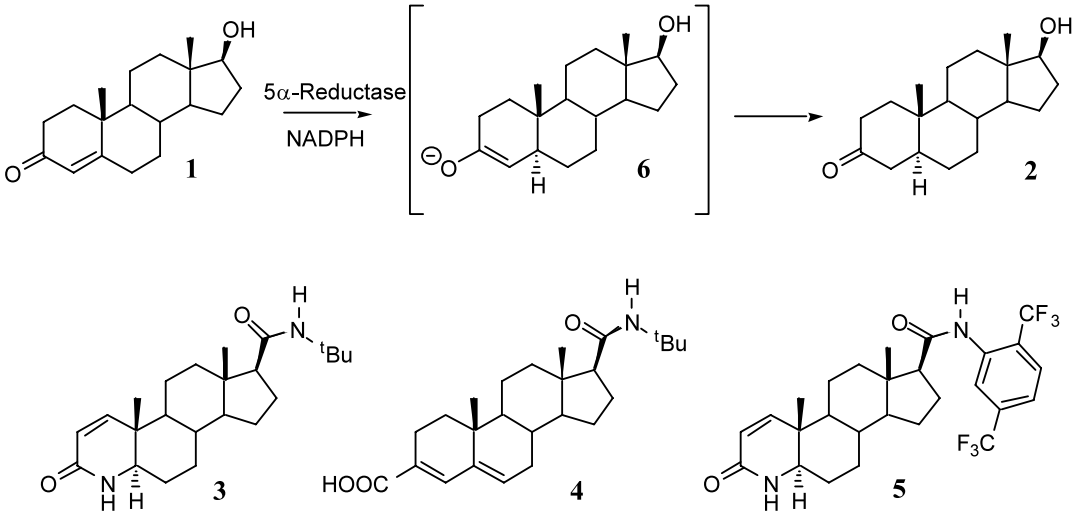
Abstract—This paper describes the rational design and synthesis of novel inhibitors of human steroid 5α-reductase. Steroidal nitrones were synthesised via an eight-step sequence from epiandrosterone and were tested for activity against type I and II 5α-reductase isozymes. Judicious placement of the nitrone into the steroid A-ring provided an effective and stable transition-state mimic of the postulated enolate intermediate involved in the conversion of testosterone into dihydrotestosterone.

Steroid 5α-reductase is the enzyme responsible for the metabolism of testosterone (**1**, T) into dihydrotestosterone (**2**, DHT) (Scheme 1).[1] Both T and DHT bind to the same androgen receptor, however the two hormones perform different physiological roles. Excessive production of DHT is considered to be a major contributive factor in many androgen-related conditions such as benign prostatic hyperplasia (BPH), acne and male pattern baldness. The notion that chemical moderation of 5α-reductase could find use in the treatment of these conditions first emerged in the early 1970s and later led to the development of several inhibitors including finasteride (MK906, Proscar®, **3**),[2] epristeride (SKF105687, **4**)[3] and dutasteride (GI198745, **5**).[4] Administration of finasteride now provides a non-surgical option for the treatment of BPH.

These inhibitors were designed to act as transition state analogues of the postulated enolate intermediate (**6**), which is generated after direct and stereospecific hydride donation from the nicotinamide cofactor NADPH to the C5 α-position of testosterone (**1**) (Scheme 1).[5] Molecular modelling of the transformation from **1** to **6** predicts considerable distortion of the



Scheme 1.

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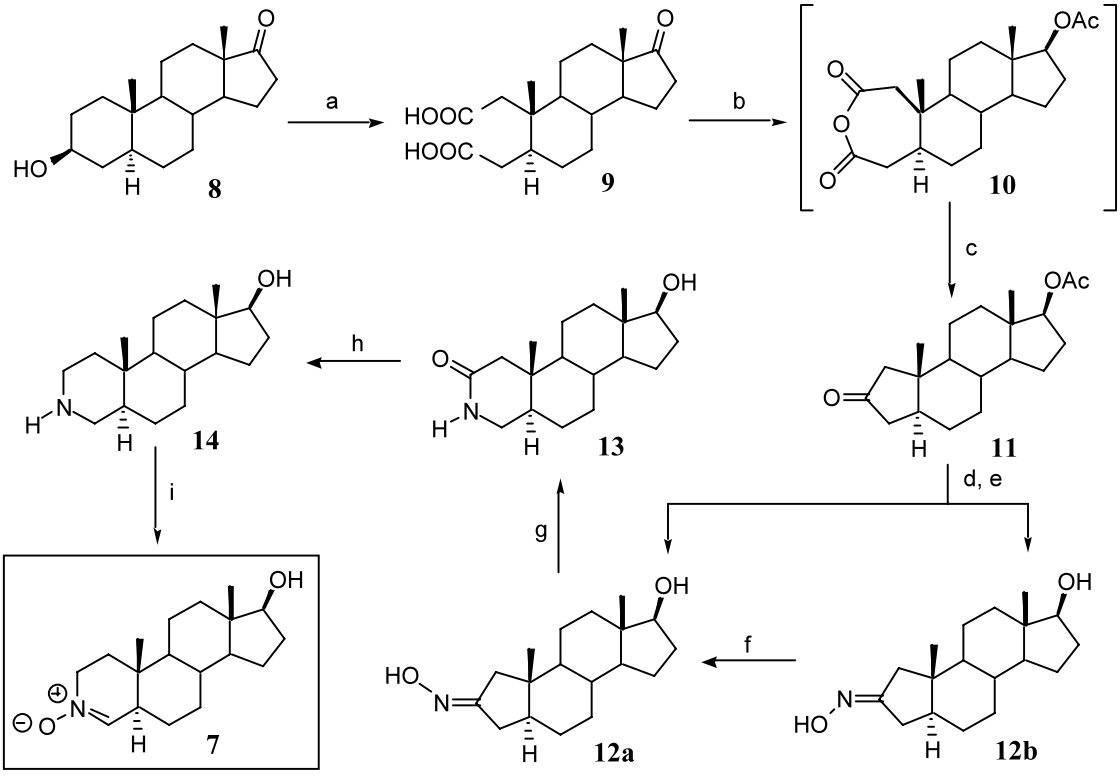
steroid A-ring; the partial chair conformation of the A-ring in testosterone is transformed into a pseudo-boat after introduction of C3–C4 sp^2-hybridisation, reorientating the C3-substituent in an upward direction. This well studied enzymatic transformation provided us with an opportunity to test the novel hypothesis that an enolate transition state might be closely mimicked by a nitrone analogue of type **7**. Indeed, molecular modelling suggested that the nitrone (**7**) would mimic the structural and polarisation features of the enolate (**6**) transition state very closely. If successful, the use of the nitrone isostere might not only led to the design of a new class of 5α-reductase inhibitor but also find application in a host of other related ketone/alcohol redox reactions.

Synthesis of inhibitors

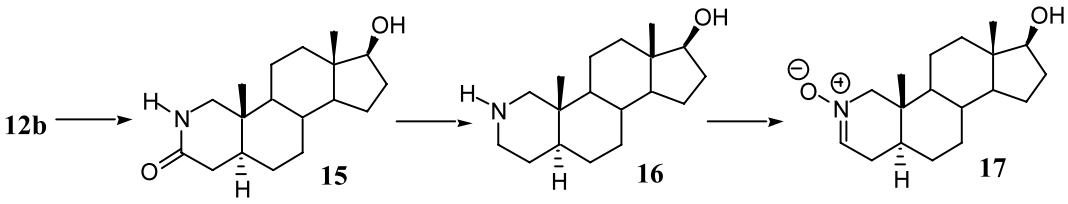
The target steroidal nitrone (**7**) was synthesised in eight steps from epiandrosterone (**8**). Insertion of the A-ring nitrogen was achieved via a sequential ring contraction–expansion strategy.[6] The A-ring was firstly oxidatively cleaved with chromium trioxide in glacial acetic acid to yield the *seco*-dicarboxylic acid (**9**) in good yield (Scheme 2). The 17-keto group was then reduced and later acetylated during the formation of the seven-membered cyclic anhydride. Vacuum pyrolysis of **10** at 260°C under reduced pressure then yielded A-nor-androsterone (**11**). Treatment of **11** with hydroxylamine hydrochloride gave an inseparable mixture of *Z*- and *E*-17β-acetoxyoximes. Hydrolysis of the acetate group provided the corresponding *Z*- and *E*-17β-hydroxy-oximes (**12a** and **12b**, 1:1) which were readily separated via column chromatography. The desired *Z*-isomer (**12a**) was found to be unstable at room temperature and slowly isomerised to give an equilibrium mixture of 2:1 *Z*- to *E*-oxime. Conveniently, the *E*-oxime (**12b**) underwent isomerisation in refluxing chloroform to give pure *Z*-isomer (**12a**). Beckmann rearrangement of **12a** to the 3-aza lactam (**13**) was performed promptly after isomerisation and eliminated the need for chromatographic separation of the oxime isomers. Reduction of **13** with lithium aluminium hydride gave amine (**14**) which was oxidised to the target nitrone (**7**) with sodium tungstate and hydrogen peroxide. A mixture of Δ^2- and Δ^3-regioisomers (1:4) was evident upon examination of NMR spectra. Although these isomers could be separated by HPLC, at room temperature they were found to slowly isomerise. Biological testing was therefore conducted on the product mixture. The *E*-oxime (**12b**) also underwent smooth rearrangement to the isomeric 2-aza lactam (**15**) which was reduced to give amine (**16**). Tungstate oxidation of **16** then yielded the analogous nitrone (**17**) also as a mixture of regioisomers (Δ^1- and Δ^2, 2:3) (Scheme 3).

Biological evaluation

Two isozymes of human 5α-reductase exist.[7] Type II predominates in the prostate and the other isozyme, designated type I, is the predominant form in facial



Scheme 2. *Reagents and conditions*: (a) CrO_3, AcOH, 60°C (70%); (b) $NaBH_4$, EtOH (75%) followed by Ac_2O, 60°C, 2 h (70%); (c) 260°C, 0.1 mmHg (70%); (d) $NH_2OH{\cdot}HCl$, EtOH, pyridine (85%); (e) KOH, MeOH, (97%); (f) $CHCl_3$, reflux, 3 h; (g) $SOCl_2$, PhH, rt (98%); (h) $LiAlH_4$, THF, reflux (92%); (i) Na_2WO_4, 30% H_2O_2, MeOH, rt (78%).



Scheme 3.

Table 1. In vitro screening of azasteroids against Type I and II human steroid 5α-reductase[a]

3-Aza series				2-Aza series			
Compound	nM	% Inhibition		Compound	nM	% Inhibition	
		Type I	Type II			Type I	Type II
14	10000	–	0	**16**	10000	–	3.6
	1000	4.2	–		1000	15.9	–
	10	–	0		10	–	0
	1	0	–		1	3.3	–
13	10000	–	56.7	**15**	10000	–	54.6
	1000	14.6	–		1000	0	–
	10	–	18.2		10	–	0.6
	1	4.0	–		1	0	–
7	10000	–	100	**17**	10000	–	70.5
	1000	73.2	–		1000	10.0	–
	10	–	27.5		10	–	31.4
	1	0	–		1	7.2	–

[a] Testing performed at pH 7.5.

skin where its activity contributes one-third of circulating levels of DHT. The development of dual type I and II inhibitors would enable complete reduction of DHT production and may be advantageous in the treatment of BPH. Significantly, in the case of finasteride (**3**), a selective type II inhibitor, residual circulating DHT can still be high following treatment.[8] Biological evaluation of the 3-aza (**7**,**13**,**14**) and 2-aza (**15**–**17**) steroids[9] for human 5α-reductase inhibition was conducted and the results are presented below (Table 1). As expected, amines **14** and **16** showed poor inhibitory activity in both the type I and II screens and the lactams **13** and **15** displayed only marginal improvement against the type II isozyme. Inhibitory data obtained for the designed nitrone (**7**), however, showed significant enhancement in biological activity. Notably, this molecule was the most potent out of the six steroids tested and showed good inhibitory activity against both isozymes.

Although not related to the mechanism of action, the nature of the C17 substituent on the steroid nucleus has been shown to dramatically affect the potency of designed inhibitors. In general, large and less polar functionalities, such as the 17β-(*N*-alkyl)-carbamoyl group found in inhibitors **3** and **4** interfere more effectively with the enzyme. It is therefore highly likely that the 17β-hydroxyl group used in this study does not convey the maximum inhibitory activity capable for the designed steroidal nitrone (**7**). Furthermore, C17 substitution of both 4- and 6-azasteroids with (arylcycloalkyl)amides and 2,5-disubstituted anilides,[10] such as that found in dutasteride (**5**), has recently been shown to yield potent inhibitors of both type I and II 5α-reductase. The future synthesis of nitrone analogues bearing bulky 17β-amide functionality may therefore lead to more potent dual inhibitors of type I and II human 5α-reductase. In conclusion, the preliminary results in this study demonstrate *the novel* principle that a nitrone can serve as a stable mimic of an enolate transition state.

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References

1. Recent reviews on 5α-reductase inhibition: (a) Bratoeff, E.; Ramirez, E.; Murillo, E.; Flores, G.; Cabeza, M. *Curr. Med. Chem.* **1999**, *6*, 1107–1123; (b) Li, X.; Chen, C.; Singh, S. M.; Labire, F. *Steroids* **1995**, *60*, 430–441.

2. Stoner, E. J. *J. Steroid Biochem. Mol. Biol.* **1990**, *37*, 375–378.
3. Holt, D. A.; Levy, M. A.; Metcalf, B. W. *Adv. Med. Chem.* **1993**, *2*, 1–29 and references cited therein.
4. Tian, G.; Mook, R. A.; Moss, M. L.; Frye, S. V. *Biochem.* **1995**, *34*, 13453–13459.
5. Bull, H. G.; Garcia-Calvo, M.; Andersson, S.; Baginsky, W. F.; Chan, H. K.; Ellsworth, D. E.; Miller, R. R.; Stearns, R. A.; Bakshi, R. K.; Rasmusson, G. H.; Tolman, R. L.; Myers, R. W.; Kozarich, J. W.; Harris, G. S. *J. Am. Chem. Soc.* **1996**, *118*, 2359–2365 and references cited therein.
6. (a) Boswell, G. A. Ph.D. Dissertation, University of California (Berkeley), 1959; (b) Djerassi, C. *Steroid Reactions–An Outline for Organic Chemists*; Holden-Day, Inc: San Francisco, 1963; (c) Rasmusson, G. H.; Reynolds, G. F.; Utne, T.; Jobson, R. B.; Primka, R. L.; Berman, C.; Brooks, J. R. *J. Med. Chem.* **1994**, *27*, 1690–1701; (d) Oka, K.; Hara, S. *Chem. Ind.* **1968**, 168–170.
7. Russell, D. W.; Wilson, J. D. *Annu. Rev. Biochem.* **1994**, *63*, 25–61.
8. Bartsch, G.; Rittmaster, R. S.; Klocker, H. *Eur. Urol.* **2000**, *37*, 367–380.
9. Nitrones **7** and **17** are novel compounds and gave IR, NMR and mass spectral data consistent with the assigned structures and satisfactory elemental analyses. All other intermediates were fully characterised.
10. Frye, S. V.; Haffner, C. D.; Maloney, P. R.; Hiner, R. N.; Dorsey, G. F.; Noe, R. A.; Unwalla, R. J.; Batchelor, K. W.; Bramson, H. N.; Stuart, J. D.; Schweiker, S. L.; van Arnold, J.; Bickett, D. M.; Moss, M. L.; Tian, G.; Lee, F. W.; Tippin, T. K.; James, M. K.; Grizzle, M. K.; Long, J. E.; Croom, D. K. *J. Med. Chem.* **1995**, *38*, 2621–2627.