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Simple Bi- and Tricyclic Inhibitors of Human Steroid 5α-Reductase

Andrew D. Abell,[a,*] Maureen J. Prince,[a] Ann M. McNulty[b] and Blake L. Neubauer[b]

[a]*Department of Chemistry, University of Canterbury, Christchurch, New Zealand*
[b]*Lilly Research Laboratories, Indianapolis, IN 46285, USA*

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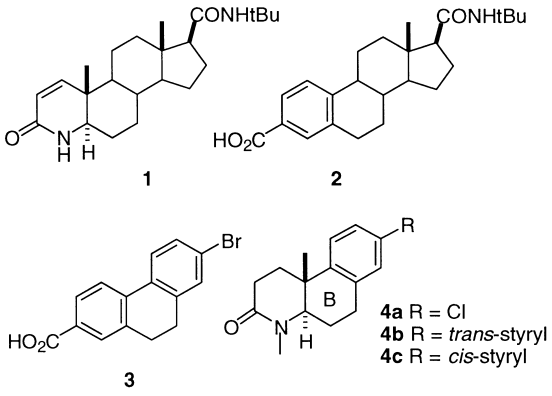
Abstract—A number of tricyclic thiolactams, bicyclic lactams, and bicyclic thiolactams have been prepared and evaluated in vitro as inhibitors of types 1 and 2 steroid 5α-reductase. The tricycles with an 8-chloro substituent in the C-ring are nM (IC_{50}) inhibitors of type 1 steroid 5α-reductase (SR). In all the cases studied, lactams are more potent than the corresponding thiolactams. Activity against type 2 SR is greatly enhanced by a styryl (or azo) substituent on the aryl ring of the tri- and bicycles and also a related tricyclic aryl acid.

A number of different classes of compounds are known to inhibit steroid 5α-reductase (SR), an NADPH-dependent enzyme that catalyses the bioreduction of testosterone to the more potent androgen, dihydrotestosterone (DHT).[1] These inhibitors have been actively pursued as potential therapeutic agents for the treatment of pharmacological disorders associated with elevated levels of DHT including benign prostatic hyperplasia (BPH),[1] some prostatic cancers,[2] skin disorders such as acne,[3] male pattern baldness,[4] and hirsutism.[5] Initial approaches to the design of inhibitors of SR employed the steroid nucleus of the natural substrate of the enzyme to provide mimics of the proposed enolate intermediate in the bioreduction.[1] This work led to inhibitors of the type **1**[6] and **2**.[7] More recently, a number of simpler, nonsteroidal, inhibitors of SR have been identified (e.g. **3**[8] and **4a**).[1b,9] A number of structural similarities between the steroid and nonsteroidal series have been noted, particularly in the A-ring functionality (c.f. **1/4** and **2/3**).[1b,8] The structural factors that influence the potency and isozyme selectivity[10] of the two series have also been studied, although such studies on the nonsteroidal compounds are still in their infancy. In this paper we begin to assess some of these factors, in particular the role of the B-ring and lactam functionality of the tricyclic analogues **4**. These issues have been addressed in the first instance with the preparation and testing, against types 1 and 2 SR, of the tricyclic thiolactams **6**, **7**, **9**, the bicyclic lactams **12**, **16**, **17**, **20**, and the thiolactam **19**. We also prepared and tested the tricyclic aryl acid **11** to provide further insight into the role of the styryl substituent in the related, tricyclic lactam series of nonsteroidal compounds (see compounds **4b** and **4c**) where this group has been observed to provide dual isozyme inhibitors.[11]



Synthesis

The tricyclic thiolactams **6**, **7** and **9** were conveniently prepared by treating the corresponding lactones **5**,[9] **4a**,[9] and **8**,[9] respectively, with [2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane (Lawesson's reagent) (Scheme 1). The tricyclic aryl acid **11** was prepared from the known aryl bromide **10**[8] by palladium mediated coupling,[11] followed by ester hydrolysis (Scheme 1). The bicyclic thiolactam **19** was prepared from **16**[12] by treatment with Lawesson's reagent, Scheme 2. Compound **17** was

*Corresponding author. Tel.: +64-3-364-2818; fax: +64-3-364-2110; e-mail: a.abell@chem.canterbury.ac.nz

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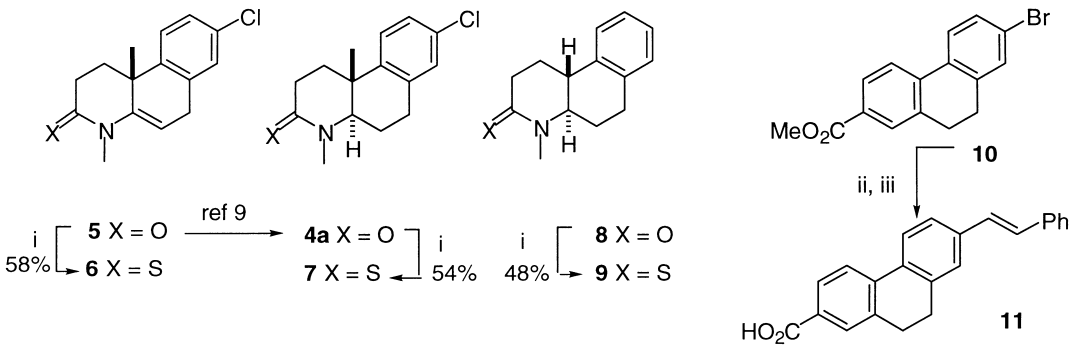
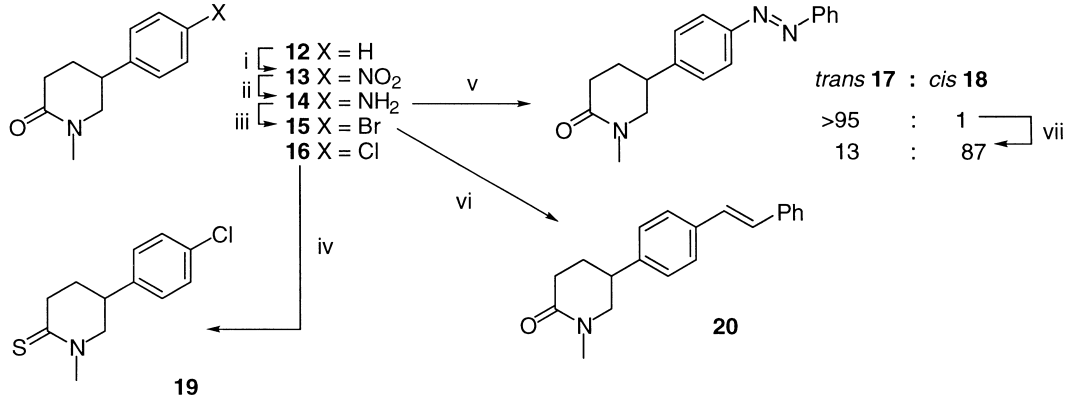


Scheme 1. Reagents and conditions: (i) Lawesson's reagent, THF (**5**, reflux 6 h; **4**, reflux 18 h; **8**, rt 30 min); (ii) styrene, $Pd(OAc)_2$, (*o*-tol)$_3$P, Et_3N, AcCN, 100 °C, 24 h; (iii) K_2CO_3, MeOH, H_2O, reflux.



Scheme 2. Reagents and conditions: (i) KNO_3, c H_2SO_4, rt (49%); (ii) PtO_2, AcOH, H_2, rt (51%): (iii) $CuBr_2$, *t*-butylnitrite, AcCN, rt (18%); (iv) Lawesson's reagent, THF, rt (87%); (v) nitrosobenzene, AcOH, rt (62%); (vi) styrene, $Pd(OAc)_2$, (*o*-tol)$_3$P, Et_3N, AcCN, 100 °C, 24 h.

prepared as a mixture (contained < 5% of the corresponding *cis*-isomer by ^{1}H NMR)[13] by coupling the aryl amine **14** with nitrosobenzene in acetic acid. Compound **14** was itself prepared from **12**[12] in a two step sequence involving nitration followed by reduction with palladium oxide and hydrogen. The aryl amine **14** also gave rise to the aryl bromide **15** and this in turn under went palladium catalyzed coupling with styrene to give the styryl bicyclic lactone **20** (Scheme 2).

Table 1. Inhibition of types 1 and 2 SR

No.	Type 1 IC_{50} (nM)	Type 2 IC_{50} (nM) or % inhibition
3[8]	26[a]	20% @ 10 μM
5[9]	120	—
6	377	13.2% @ 40 μM
4a[9]	17	—
4b[11]	23	180
7	183	21.6% @ 40 μM
8[9]	560	—
9	1450	19.9% @ 40 μM
11	152	340
12	2477	13.5% @ 40 μM
16	1690	12,350
17	302	579
19	3360	14% @ 40 μM
20	107	617

[a] $K_{i,app}$

Enzyme Inhibition

The IC_{50} values (or % inhibition at constant concentration for the less active compounds) were determined against types 1 and 2 SR[14] and the results are given in Table 1. An analysis of these results reveals some important trends. First, the tricyclic thiolactams **6** and **7** are, like their lactam analogues,[9] selective for type 1 SR. However, in all cases thiolactams are less active than the corresponding lactams (c.f. **5/6**, **4a/7** and also **16/19**). A chloro substituent in the C-ring is favored for type 1 activity in both series (c.f. **4a/8** and **7/9**) as is the absence of a double bond in the B-ring (c.f. **5/4a** and **6/7**). It should be noted that a steroid-based thiolactam has been reported to be an inhibitor of rat prostatic SR.[15]

The aryl acid **11** shows good dual isozyme inhibitory properties with significantly enhanced type 2 activity compared to previously reported compounds of this type (e.g. **3**; type 1 $K_{i,app} = 26$ nM, type 2 gave 20% inhibition at 10 μM).[8] However, **11** is still less active than the equivalent bicyclic lactam **4b**[11] (IC_{50} type 1 = 23 nM, type 2 = 180 nM).

Bicyclic lactams, in general, would appear to be less active against type 1 SR than the tricycles (c.f. **4a/16**). In contrast to the tricycles, a chloro substituent is not optimum optimum for type 1 activity (c.f. **12**, **16**, and

20), however as before, a thiolactam produces a compound with decreased type 1 activity as compared to a lactam (c.f. **16/19**). The results for **17** and **20** reinforce an important, and apparently general observation that a styryl (or azo) substituent dramatically enhances type 2 activity (and indeed type 1 activity with the bicycles). Substituents of this type would appear to provide dual inhibitors of types 1 and 2 SR in all series studied, namely tricyclic lactams, aryl acids and bicyclic lactams. It should also be noted that some bicyclic aryl acids have been reported to be type 2 selective.[16]

In conclusion, a number of tricyclic thiolactams, bicyclic lactams and bicyclic thiolactams have been prepared and evaluated in vitro as inhibitors of types 1 and 2 steroid 5α-reductase. We have shown that similar substituent effects operate throughout these compounds and also the tricyclic aryl acids **3** and **11**. It should also be noted that the opportunity exists to photoswitch **17** between its *trans* and *cis* forms as a potential means to modulate its types 1 and 2 activity.[17] Finally, compounds **11**, **17** and **20** are good dual isozyme inhibitors in spite of their simple structure relative to previously reported inhibitors of SR.[1] Even more potent compounds of this type should be able to be prepared by further fine-tuning the substituents.

Acknowledgements

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13. Photolysis of this mixture gave a 13:87 mixture of **17** and **18**.
14. Type 1 assays: The conversion of ^{3}H-labelled T into ^{3}H-labelled DHT using type 1 SR isolated from human scalp was measured as previously described (see McNulty, A. M.; Audia, J. E.; Bemis, K. G.; Goode, R. L.; Rocco, V. P.; Neubauer, B. L. *J. Steroid Biochem. Mol. Biol.* **2000**, *72*, 13). In short, the test compounds were dissolved in methanol to 10, 100, 1000, and 10,000 nM concentrations. Labeled T (80 nM, 8.07×10^{-3} μCi specific activity 92.41 Ci/mmol) and unlabelled T (0.92 μM) were combined with the test compounds to give a total substrate concentration of 1.0 μM in a volume of 200 μL. The assay was initiated by the addition of 0.015–0.025 mg of type 1 SR homogenate to the substrate and the mixture was then incubated for 1 h at 37 °C. Substrates and metabolites were quantified by HPLC separation and in-line flow radioactivity detection of the labelled androgens.
Type 2 assay: This was carried out using SR isolated from human prostate tissues (11.34 mg per reaction). T was added (50 nM, specific activity 40.3 Ci/mmol) and the mixture was incubated at 25 °C for 1 h. The inhibitors were prepared and tested as described for the type 1 assay. A type 1 specific inhibitor was analysed for activity against the type 2 enzyme preparation and it showed no detectable inhibition at 1 μM concentration. The activity of both assays was calibrated using known type 1 and type 2 inhibitors.
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17. In support, compounds **4b** and **4c** show different isozyme selectivity (see ref 11).