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A COMPARISON OF STEROIDAL AND NON-STEROIDAL INHIBITORS OF HUMAN STEROID 5α-REDUCTASE: NEW TRICYCLIC ARYL ACID INHIBITORS OF THE TYPE-1 ISOZYME

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Abstract: A series of 9,10-dihydrophenanthrene-2-carboxylic acids has been prepared and evaluated *in vitro* as inhibitors of human recombinant steroid 5α-reductase. 7-Bromo-9,10-dihydrophenanthrene-2-carboxylic acid, **8c**, is a potent and selective non-steroidal inhibitor of human type-1 steroid 5α-reductase ($K_{i,app}$ 26 nM). The inhibitory activity relationships of steroidal and non-steroidal inhibitors, with 4-aza, 6-aza, diene acid, aryl acid and nitro-alkenes functionalities, are considered.

Considerable effort has gone into the design and preparation of inhibitors of steroid 5α-reductase (SR), an NADPH-dependent enzyme that reduces testosterone to dihydrotestosterone (DHT).[1] SR inhibitors offer potential as therapeutic agents for the treatment of pharmacological disorders associated with elevated levels of DHT such as benign prostatic hyperplasia (BPH),[1] some prostatic cancers,[2] skin disorders such as acne,[3] male pattern baldness,[4] and hirsutism.[5] Two isozymes of steroid 5α-reductase, differing in their pattern of tissue distribution and with distinct biochemical and pharmacological properties, have recently been identified.[6] Finasteride, a 4-azasteroid currently marketed as a treatment for BPH, is selective for the type-2 isozyme which is the predominant form in this human tissue (type-1 IC_{50} = 500nM, type-2 IC_{50} = 4.2nM).[1,7] A number of other steroid-based inhibitors of SR are also potent against the type-2 isozyme (Table 1) although recent studies[8] have shown that the type-1 potency can be enhanced with the correct choice of the C-17 substituent (compare **3a** and **3b**, Table 1). Localization of type-1 SR in the skin has led to the suggestion that selective inhibitors of this isozyme activity could provide therapies for acne, male pattern baldness, and hirsutism.

A series of benzoquinolinones, typified by **2a** and **2b**, also has been identified as providing potent type-1, non-steroidal, inhibitors of SR.[9] Based on the structural similarity between the 4-azasteroid SR inhibitors, e.g., **1**, and the benzoquinolinones, e.g., **2a** and **2b**, we prepared the non-steroidal diene acids **6a** and **6b**.[10] These compounds represent non-steroidal analogs of the steroid-based diene acid SR inhibitors **5a** and **5b**, respectively. The analogy between the steroidal and non-steroidal series was further investigated with the preparation and evaluation of **4**, an analog of the 6-azasteroids **3**, as an inhibitor of the two SR isozymes.[11] The structurally related derivatives **2** and **4** are selective for the type-1 isozyme of SR with compounds of the type **2** having significantly greater potency than **4**. By contrast, compounds **6** are selective inhibitors of type-2 SR. Inhibitory potency is increased by the presence of an 8-chloro- substituent in all three series. Here, we report the synthesis and testing of the tricyclic aryl acids **8a-c**, **9** and the tricyclic nitro-alkene **11** as non-steroidal analogs of the steroid-based, type-2 selective, SR inhibitors **7** and **10**, respectively.

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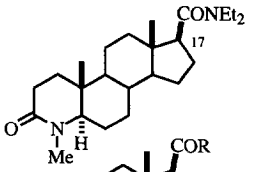
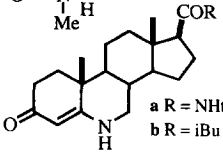
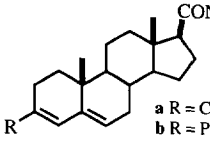
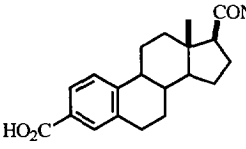
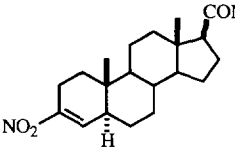
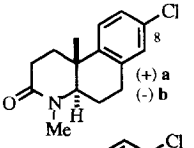
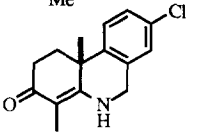
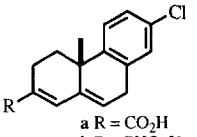
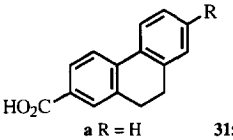
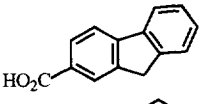
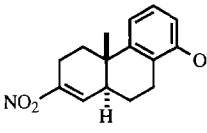
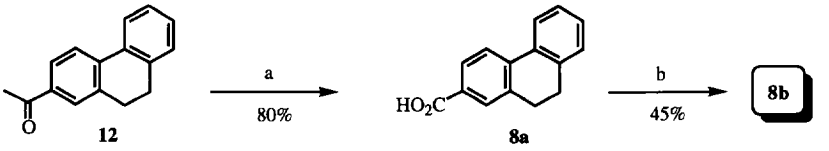
Chemistry.[12] The tricyclic aryl acid **8a**, prepared from **12**,[13] was treated with chlorine and trimethyl phosphate, according to the general literature procedure,[14a] to give **8b** (Scheme 1). Reaction of **13** with bromine and trimethyl phosphate[14a] gave the dibromide **14** which under went a high pressure carbonylation in methanol[14b] to give the methyl ester **15** (Scheme 2). Hydrolysis of the methyl ester of **15** gave the required bromo aryl acid **8c**. The nitro-alkene **11** was prepared as a racemate from the enone **16**[15] (Scheme 3) by a sequence analogous to that reported for the preparation of **10**.[16] Lithium in ammonia reduction of **16**, followed by trapping of the resulting enolate with *N*-phenyltrifluoromethanesulfonyl chloride, gave the triflate **17**. Conversion of **17** to the stannane **18** was followed by nitration with tetranitromethane to give **11**.

Table 1. Inhibition of recombinant types-1 and 2 human steroid 5α-reductase.

No.[a]	Compound	$K_{i,app}$ (nM) type -1	type-2
1[7,17]		3	2-3
3[8]	 a R = NHtBu b R = iBu	820[b] 9[b]	0.89[b] 0.08[b]
5[17-19]	 a R = CO_2H b R = PHO_2H	410 3200	0.2 1.3
7[18]		1600	0.4
10[16]		4200	30-50
2[9]	 (+) a (-) b	9 10	NI[c] NI[c]
4[11]		920[b]	~20000[b,d]
6[10]	 a R = CO_2H b R = PHO_2H	1200 1900	260 1600
8	 a R = H b R = Cl c R = Br	315 320 26	>10000[e] ~2500[f] 10000
9		>2500[g]	10000
11		>2500[h]	NI[c]

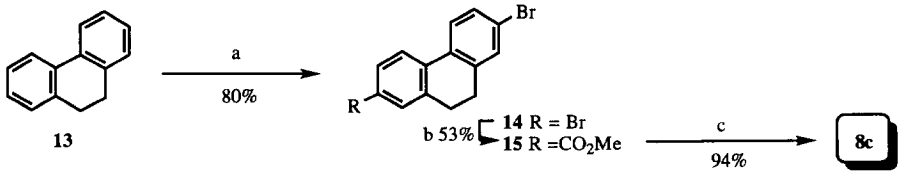
[a] literature reference
[b] not SmithKline Beecham data (see ref.)
[c] no inhibition observed at 1 μM
[d] 49% inhibition at 20 μM
[e] 20% inhibition at 10 μM
[f] 50% inhibition at 2.5 μM
[g] 30% inhibition at 2.5 μM
[h] 10% inhibition at 2.5 μM

Scheme 1



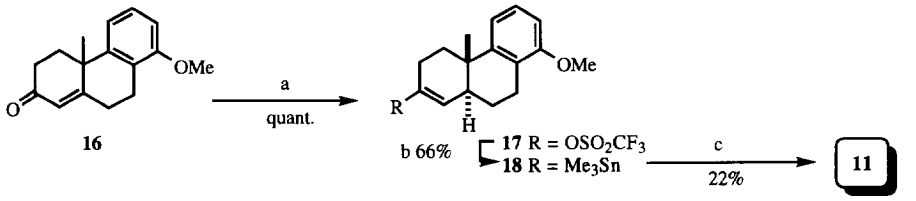
Conditions: (a) A solution of Br_2, NaOH in H_2O was added to **12** in dioxane, 30 °C, 0.5 h (see ref 14a); (b) Cl_2, Me_3PO, 45-100 °C, 1.5 h.

Scheme 2



Conditions: (a) Br_2, Me_3PO, 18 °C for 18 h then -15 °C for 48 h; (b) $PdCl_2$, Ph_3P, Et_3N, MeOH/benzene (1:2.1), CO (575 psi), 150 °C for 4 h; (c) K_2CO_3, MeOH/H_2O (10:1), reflux 18 h.

Scheme 3



Conditions: (a) Li in NH_3, aniline, THF, -78 °C, 2 h then isoprene followed by $(CF_3SO_2)_2NC_6H_5$, THF, 5-18 °C over 18 h; (b) $(Me_3Sn)_2$, $(Ph_3P)_4Pd$, LiCl, THF 60 °C, 18 h; (c) $C(NO_2)_4$, DMSO/CCl_4 (1:1), 18 °C, 2 h followed by 75 °C, 18 h.

Enzyme Inhibition. The apparent inhibition constants ($K_{i,app}$) were determined for compounds **8a-c**, **9** and **11** using recombinant type-1 and type-2 human steroid 5α-reductases as described.[17] The tricyclic aryl acids **8a-c** show selective inhibition of the type-1 isozyme of SR with the 8-bromo substituent providing the most potent inhibitor (type-1 isozyme inhibition constants for **8a-c** were 315, 320 and 26 nM, respectively, Table 1). The type-1 selectivity of **8a-c** is consistent with that observed for the non-steroidal derivatives **2** and **4** but is opposite to that observed for the diene acids **6**. Type-2 selectivity has also been observed for benzophenone carboxylic acid and indole carboxylic acid non-steroidal inhibitors of SR.[20] A six-membered B-ring appears to be a requirement for the most favorable type-1 inhibition, as can be observed by comparing the results with compounds **8a** and 9H-fluorene-2-carboxylic acid **9** (Table 1). The nitro-alkene **11** is only a weak inhibitor of type-1 SR (inhibition constant of >2500 nM, Table 1). However, it should be noted that a 7-methoxy substituent is probably not the optimum substituent for activity. Of the five classes of tricyclic non-steroidal inhibitors studied to date, typified by compounds **2**, **4**, **6**, **8** and **11**, all but the diene acids **6** are selective for the type-1 isozyme of SR. A halogen substituent at the 8-position would also appear to be favourable for inhibition of type-1 SR activity.[9-11] The benzoquinolinone non-steroidal inhibitors **2** and the aryl acid inhibitors **8** (summarized in Table 1) represent the best inhibitors of type-1 SR within this group of tricyclic compounds. In summary, potent non-steroidal inhibitors of SR, based on the tricyclic skeleton initially identified in compounds **2**, are obtained by employing A-ring groups (4-aza, 6-aza, diene acid, aryl acid and nitro-alkene) that have been used to yield potent steroid-based inhibitors of SR.

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