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PREPARATIVE CHIRAL HPLC SEPARATION OF ALL POSSIBLE STEREOISOMERS OF LY191704 AND LY266111 AND THEIR *IN VITRO* INHIBITION OF HUMAN TYPES 1 AND 2 STEROID 5α-REDUCTASES

Andrew D. Abell*

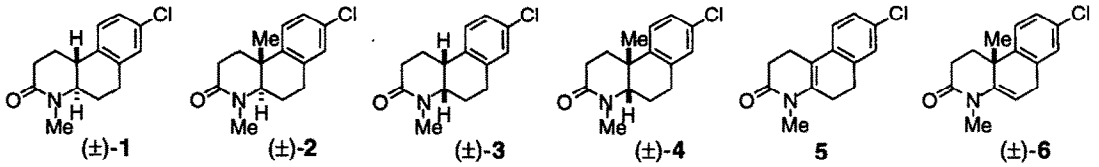
Department of Chemistry, University of Canterbury, Christchurch, New Zealand

Karl F. Erhard, Hwa-Kwo Yen, Dennis S. Yamashita, Martin Brandt, Hadiza Mohammed, Mark A. Levy, and Dennis A. Holt*

Department of Medicinal Chemistry, SmithKline Beecham Pharmaceuticals
P.O. Box 1539, King of Prussia, Pennsylvania 19406-0939

Abstract: A preparative chiral HPLC separation of each of the four stereoisomers of LY191704 [(±)-**1**, and (±)-**3**] and LY266111 [(±)-**2**, and (±)-**4**] is reported. All eight compounds have been evaluated *in vitro* as inhibitors of recombinant human type 1 and type 2 steroid 5α-reductase. The *trans* enantiomers of LY266111, (+)-**2** and (-)-**2**, show equal and potent inhibition of the type 1 isozyme. The *cis* enantiomers of LY266111, (+)-**4** and (-)-**4**, and the unsaturated analogue **6** show significantly reduced type 1 inhibitory activity. The *cis* and *trans* enantiomeric pairs of LY191704 [(+)-**1**, (-)-**1**, (+)-**3**, and (-)-**3**] and the unsaturated analog **5** display similar and potent activity against the type 1 isozyme. All compounds display relatively poor activity against the human type 2 isozyme.

Dihydrotestosterone (DHT) is a potent androgenic steroid implicated in the trophic support of tissues such as the prostate and skin. DHT is produced from the stereospecific reduction of testosterone, a reaction catalyzed by the NADPH-dependent enzyme steroid 5α-reductase (EC 1.3.99.5). The inhibition of steroid 5α-reductase provides potential therapeutic intervention of disorders such as benign prostatic hyperplasia (BPH)[1], prostatic cancer[2] and skin disorders including acne,[3] male pattern baldness,[4] and hirsutism.[5] The existence of two isozymes of steroid 5α-reductase encoded by distinct genes has recently been demonstrated.[6] The two isozymes have different patterns of tissue distribution and distinct biochemical and pharmacological properties. Several classes of steroidal inhibitors of steroid 5α-reductase have been reported.[7-10] A few non-steroidal inhibitors of steroid 5α-reductase have also been reported including ONO-3805[11] and more recently a series of benzoquinolinones,[12,13] typified by LY191704 (**1**) and LY266111 (**2**). The benzoquinolinone compounds have been reported to be specific inhibitors of human type 1 steroid 5α-reductase.[12,13]



(±)-**1** (±)-**2** (±)-**3** (±)-**4** **5** (±)-**6**

Racemic LY191704 (**1**) and LY266111 (**2**) are readily prepared from substituted 2-tetralones.[12] The final step in the synthesis requires the separation of (**1**) and (**2**) from the corresponding *cis* isomers (**3**) and (**4**),

respectively. The individual enantiomers of LY191704 (**1**) and LY266111 (**2**) have also been prepared by means of separation of diastereomeric intermediates.[14] The IC_{50} values have been reported for racemic LY191704 (**1**) and LY266111 (**2**) with type 1 steroid 5α-reductase in cultured cells.[12] Herein we report a convenient, preparative HPLC separation of the 8 stereoisomers **1**, **2**, **3** and **4**, and the apparent inhibition constants ($K_{i,app}$) for each stereoisomer using recombinant type 1 and type 2 human steroid 5α-reductases.

A mixture[12] of (±)-**2** and (±)-**4** was separated, using a preparative DIACEL CHIRALPAK AS column,[15] to give (+)-**2**, (-)-**2**, (+)-**4** and (-)-**4** in >95% ee[16] The same chromatographic conditions[15] also provided (+)-**1**, (-)-**1**, (+)-**3** and (-)-**3**, in >95% ee.[17] Gram quantities of the major (*trans*) stereoisomers can be conveniently obtained in each series. The apparent inhibition constants ($K_{i,app}$)[18-20] for each enantiomer of **1**, **2**, **3** and **4**, and for the synthetic precursors **5** and racemic **6**, are reported in Table 1. In our assay, the angularly unsubstituted *trans* enantiomers, (+)-**1** and (-)-**1**, and the angularly substituted *trans* enantiomers, (+)-**2** and (-)-**2**, show very similar and potent inhibition of the type 1 isozyme (Table 1). Compound **5**, and the angularly unsubstituted *cis* enantiomers, (+)-**3** and (-)-**3**, also show very potent inhibition of the type 1 isozyme (Table 1). By contrast, racemic **6** and the angularly substituted *cis* enantiomers, (+)-**4** and (-)-**4**, show very much reduced type-1 activity.

Table 1. Inhibition of Type 1 and 2 Steroid 5α-Reductases

Compd	$K_{i,app}$ (nM) type 1	type 2	Compd	$K_{i,app}$ (nM) type 1	type 2
(+)-**1**	**6**	**1000**	(+)-**2**[a]	**9**	**>1000**
(-)-**1**[a]	**4**	**1200**	(-)-**2**	**10**	**>1000**
(+)-**3**[b]	**15**	**1000**	(+)-**4**[b]	**4000**	**>2500**
(-)-**3**[b]	**15**	**5400**	(-)-**4**[b]	**7000**	**>2500**
5	**17**	**455**	(±)-**6**	**180**	**NI**[c]

[a] absolute configuration as drawn on first page (see ref 13).
[b] absolute configuration unknown.
[c] no inhibition observed at 1μM.

A conformational search[21] of the *trans* derivatives (**1**) and (**2**) revealed two low energy conformations of essentially identical energies, differing primarily in the conformation of the A-ring (half chair vs boat). Similar conclusions can be drawn using either conformation, however, the more linear half chair conformation is used in the following analysis. Figure 1 depicts the result of overlaying the A and C rings of the *trans* enantiomers of **2**, such that the angular methyl groups coincide. An alternative exact overlay of the A, B and C rings of (+)-**2** and (-)-**2** (not shown) places the angular methyl groups on opposite faces. The excellent overlay of the ring systems and 8-chloro substituent of (+)-**2** and (-)-**2** may account for the observed similar and potent activity of the *trans* enantiomers.[22] The 8-chloro substituent is known to be critical for the potency of the benzoquinolinone compounds against human type 1 steroid 5α-reductase.[12] Similar conclusions can be drawn for (+)-**1** and (-)-**1** using a structural overlay analogous to that shown for (+)-**2** and (-)-**2** in Figure 1, or, in this case where the orientation of an angular methyl group is not a consideration, an exact overlay of the A, B and C rings (Figure 2). The extended planar nature of the *trans* compounds, in both series, permits an excellent overlay of the enantiomeric pairs. As might be expected, the planar unsaturated analog (**5**) also displays potent type 1 inhibitory activity (Table 1).

The double bond in the angularly methyl-substituted compound (**6**) results in a slightly bowl shaped structure. The loss of planarity may account for the reduced type 1 activity of this compound relative to **2** (Table 1). The *cis* enantiomers, (+)-**4** and (-)-**4** show a dramatically reduced type 1 potency. A conformational

search[21] of the *cis* derivatives (**4**) revealed two low energy conformations, differing primarily in the conformation of the half chair B-ring, and possessing a pronounced bowl shaped structure. Figure 3 depicts the result of overlaying the A ring of each of the *cis* conformations of **4** with one of the *trans* enantiomers, (-)-**2**. The angular methyl group is again used as a common reference point for alignment of the A-rings. In both conformations of **4**, the *cis* ring junction decreases the overall planarity of the molecule resulting in a poor overlay of the B and C rings and the 8-chloro groups of the *cis* and the *trans* isomers.

In contrast to **4**, the angularly unsubstituted *cis* compounds (+)-**3** and (-)-**3** show similar and potent inhibition of the type 1 isozyme (Table 1). This perhaps unexpected result can be rationalized by considering an overlay of structures of **1** and the most planar conformation[23] of **3**, an example of which is shown in Figure 4. The absence of the constraints of the angular methyl group permits a good overlap of the A-ring and the 8-chloro substituent of the *cis* and *trans* stereoisomers. This may allow a common mode of binding to the enzyme and hence explain the potent inhibition of type 1 steroid 5α-reductase. All of the compounds tested display relatively poor activity against the type 2 isozyme (Table 1).

In summary, compounds **1**, **2** and **5** have extended planar structures and display potent inhibition of human type 1 prostatic steroid 5α-reductase. Some disruption of planarity can be tolerated without the loss of inhibitory activity as long as the angular position is unsubstituted as in compounds **3** but not **4** or **6**.

Figure 1. Red, (-)-(**2**); Black, (+)-(**2**)

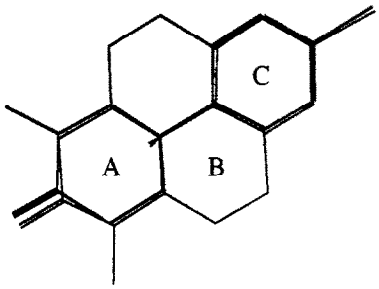


Figure 2. Red, (+)-(**1**); Black, (-)-(**1**)

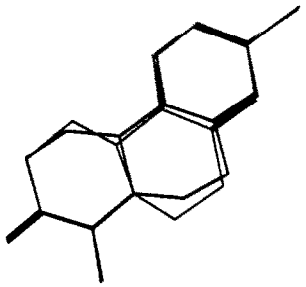


Figure 3. Red, (-)-(**2**); Blue and Green, (**4**)

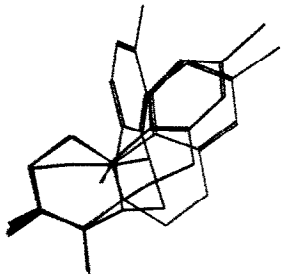
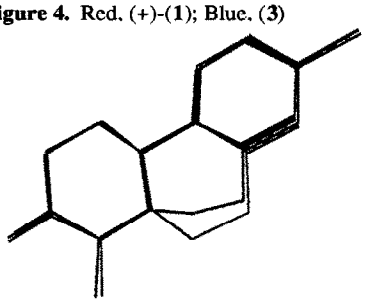


Figure 4. Red, (+)-(**1**); Blue, (**3**)



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15. Analytical HPLC: Hexane/Ethanol (80/20) on a DIACEL CHIRALPAK AS column (4.6x250 mm) at 1.0 mL/min and 210 nm detection. Preparative HPLC: Hexane/Ethanol (80/20) on a DIACEL CHIRALPAK AS column (21.2x250 mm) at 10 mL/min and 254 nm detection.
16. Order of elution (elution times): (+)-**2** (14.4 min), (+)-**4** (18.3 min), (-)-**4** (26.9 min), (-)-**2** (30.5 min). $[\alpha]^{23}D$ ($CHCl_3$): (+)-**2** (+83°), (+)-**4** (+126°), (-)-**4** (-124°), (-)-**2** (-82°).
17. Order of elution (elution times): (-)-**1** (10.8 min), (-)-**3** (11.4 min), (+)-**1** (18.2 min), (+)-**3** (18.4 min). $[\alpha]^{23}D$ (MeOH): (-)-**1** (-79°), (-)-**3** (-87°), (+)-**1** (+76°), (+)-**3** (+81°).
18. Evaluation of steroid 5α-reductase activity was performed with recombinant human prostatic enzyme preparations as previously described.[19] Apparent inhibition constants ($K_{i,app}$) of test compounds were determined by the method of Dixon.[20]
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21. Low energy conformational space was searched using the Monte Carlo routine of Macromodel with an MM2 force field.
22. This overlap hypothesis results in a translocation of the lactam bond (which presumably mimics the 3,4-steroidal enolate transition state by virtue of its geometry[7]) into the 2,3 position (steroid numbering). However, in this benzoquinolinone, the calculated O=C-C-C torsion angle is 172° and thus should also serve as a reasonable mimic for the enolate double bond. Perhaps more significant in this overlay model is the change in the location of the N-Me group within the binding site of the enzyme. A divergence of SAR for the nitrogen substituent would serve to support this model.
23. A conformational search on **3** produced the same two conformations discussed for **4**.

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